09/882,509

(FILE 'HOME' ENTERED AT 17:11:46 ON 29 DEC 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005

```
L1         42118 S STREPTOKINASE?
L2           630 S "SKC" OR "SKC-2"
L3         42539 S L1 OR L2
L4       7454183 S CLON? OR EXPRESS? OR RECOMBINANT
L5          4154 S L3 AND L4
L6          1362 S EQUISIMILIS
L7           220 S L5 AND L6
L8         49433 S INCLUSION (W) BOD?
L9             5 S L7 AND L8
L10            1 DUP REM L9 (4 DUPLICATES REMOVED)
L11          106 DUP REM L7 (114 DUPLICATES REMOVED)
L12            5 S LAMNDA
L13            1 S L11 AND INCLUSION
L14            0 S L11 AND AGGREGAT?
L15            0 S L11 AND INSOLUBLE
L16            1 S L11 AND SOLUBL?
L17           71 S L11 AND COLI
                 E KUPPUSAMY M/AU
L18           40 S E3
                 E ELLA K/AU
                 E KHATRI G S/AU
L19           46 S E3
                 E LAHIRI S/AU
L20         1660 S E3
                 E SRINIVAS V K/AU
L21           28 S E3
L22         1842 S L17 OR L18 OR L19 OR L20 OR L21
L23           71 S L3 AND L22
L24            1 S L8 AND L23
L25           71 S L4 AND L23
L26           71 S L6 AND L25
L27            1 S L26 AND L8

=>
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PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2


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 NEWS  1           Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2           "Ask CAS" for self-help around the clock
 NEWS  3  SEP 09   ACD predicted properties enhanced in REGISTRY/ZREGISTRY
 NEWS  4  OCT 03   MATHDI removed from STN
 NEWS  5  OCT 04   CA/CAplus-Canadian Intellectual Property Office (CIPO) added
                   to core patent offices
 NEWS  6  OCT 13   New CAS Information Use Policies Effective October 17, 2005
 NEWS  7  OCT 17   STN(R) AnaVist(TM), Version 1.01, allows the export/download
                   of CAplus documents for use in third-party analysis and
                   visualization tools
 NEWS  8  OCT 27   Free KWIC format extended in full-text databases
 NEWS  9  OCT 27   DIOGENES content streamlined
 NEWS 10  OCT 27   EPFULL enhanced with additional content
 NEWS 11  NOV 14   CA/CAplus - Expanded coverage of German academic research
 NEWS 12  NOV 30   REGISTRY/ZREGISTRY on STN(R) enhanced with experimental
                   spectral property data
 NEWS 13  DEC 05   CASREACT(R) - Over 10 million reactions available
 NEWS 14  DEC 14 2006 MeSH terms loaded in MEDLINE/LMEDLINE
 NEWS 15  DEC 14 2006 MeSH terms loaded for MEDLINE file segment of TOXCENTER
 NEWS 16  DEC 14 CA/CAplus to be enhanced with updated IPC codes
 NEWS 17  DEC 16 MARPATprev will be removed from STN on December 31, 2005
 NEWS 18  DEC 21 IPC search and display fields enhanced in CA/CAplus with the
                 IPC reform
 NEWS 19  DEC 23 New IPC8 SEARCH, DISPLAY, and SELECT fields in USPATFULL/USPAT2

 NEWS EXPRESS  DECEMBER 02 CURRENT VERSION FOR WINDOWS IS V8.01,
               CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 02 DECEMBER 2005.
               V8.0 USERS CAN OBTAIN THE UPGRADE TO V8.01 AT
               http://download.cas.org/express/v8.0-Discover/

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FILE 'HOME' ENTERED AT 17:11:46 ON 29 DEC 2005

=> file medline embase biosis biotechds scisearch hcaplus ntis lifesci
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'MEDLINE' ENTERED AT 17:12:17 ON 29 DEC 2005

FILE 'EMBASE' ENTERED AT 17:12:17 ON 29 DEC 2005
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FILE 'BIOSIS' ENTERED AT 17:12:17 ON 29 DEC 2005
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FILE 'NTIS' ENTERED AT 17:12:17 ON 29 DEC 2005
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FILE 'LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005
COPYRIGHT (C) 2005 Cambridge Scientific Abstracts (CSA)


=> s streptokinase?
L1       42118 STREPTOKINASE?

=> s "skc" or "SKC-2"
L2         630 "SKC" OR "SKC-2"

=> s l1 or l2
L3       42539 L1 OR L2

=> s clon? or express? or recombinant
L4     7454183 CLON? OR EXPRESS? OR RECOMBINANT

=> s l3 and l4
L5        4154 L3 AND L4

=> s equisimilis
L6        1362 EQUISIMILIS

=> s l5 and l6
L7         220 L5 AND L6

=> s inclusion (w) bod?
L8       49433 INCLUSION (W) BOD?

=> s l7 and l8
L9           5 L7 AND L8

=> dup rem l9
PROCESSING COMPLETED FOR L9
```

L10 1 DUP REM L9 (4 DUPLICATES REMOVED)

=> d all

L10 ANSWER 1 OF 1 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 1
AN 2000:96119 BIOSIS
DN PREV200000096119
TI Two **streptokinase** genes are **expressed** with different solubility in Escherichia coli W3110.
AU Pupo, Elder [Reprint author]; Baghbaderani, Behnam A.; Lugo, Victoria; Fernandez, Julio; Paez, Rolando; Torrens, Isis
CS Biopharmaceutical Development Division, Center for Genetic Engineering and Biotechnology, Havana, Cuba
SO Biotechnology Letters, (Dec., 1999) Vol. 21, No. 12, pp. 1119-1123. print.
CODEN: BILED3. ISSN: 0141-5492.
DT Article
LA English
ED Entered STN: 15 Mar 2000
Last Updated on STN: 3 Jan 2002
AB The **streptokinase** (SK) gene from S. **equisimilis** H46A (ATCC 12449) was **cloned** in E. coli W3110 under the control of the tryptophan promoter. The **recombinant** SK, which represented 15% of total cell protein content, was found in the soluble fraction of disrupted cells. The solubility of this SK notably differed from that of the product of the SK gene from S. **equisimilis** (ATCC 9542) which had been **cloned** in E. coli W3110 by using similar **expression** vector and cell growth conditions, and occurred in the form of **inclusion bodies**.
CC Genetics of bacteria and viruses 31500
Biochemistry methods - Nucleic acids, purines and pyrimidines 10052
Biochemistry methods - Proteins, peptides and amino acids 10054
Replication, transcription, translation 10300
Biophysics - Molecular properties and macromolecules 10506
Microbiological apparatus, methods and media 32000
Food microbiology - General and miscellaneous 39008
Enzymes - General and comparative studies: coenzymes 10802
Metabolism - Proteins, peptides and amino acids 13012
Morphology and cytology of bacteria 30500
Physiology and biochemistry of bacteria 31000
IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Molecular Genetics (Biochemistry and Molecular Biophysics)
IT Chemicals & Biochemicals
amino acids; enzymes; proteins; tryptophan
IT Miscellaneous Descriptors
biotechnology; cell growth conditions; **expression** vectors; gene **expression**; promoters; tryptophan promoter
ORGN Classifier
Enterobacteriaceae 06702
Super Taxa
Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria; Microorganisms
Organism Name
Escherichia coli: W 3110
Taxa Notes
Bacteria, Eubacteria, Microorganisms
ORGN Classifier
Gram-Positive Cocci 07700
Super Taxa
Eubacteria; Bacteria; Microorganisms
Organism Name
Streptococcus **equisimilis**
Taxa Notes

```
        Bacteria, Eubacteria, Microorganisms
RN   54-12-6Q (tryptophan)
     73-22-3Q (tryptophan)


=> d his

     (FILE 'HOME' ENTERED AT 17:11:46 ON 29 DEC 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005
L1         42118 S STREPTOKINASE?
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L8         49433 S INCLUSION (W) BOD?
L9             5 S L7 AND L8
L10            1 DUP REM L9 (4 DUPLICATES REMOVED)

=> dup rem l7
PROCESSING COMPLETED FOR L7
L11          106 DUP REM L7 (114 DUPLICATES REMOVED)

=> s lamnda
L12            5 LAMNDA

=> d 1-106 ibib ab

L12  ANSWER 1 OF 5     MEDLINE on STN
ACCESSION NUMBER:    77076903     MEDLINE
DOCUMENT NUMBER:     PubMed ID: 794695
TITLE:               A ribosomal RNA gene of Escherichia coli (rrnD) on
                     lamnda daro E specialized transducing phages.
AUTHOR:              Jorgensen P
SOURCE:              Molecular & general genetics : MGG, (1976 Aug 2) 146 (3)
                     303-7.
                     Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY:        GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE:       Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
FILE SEGMENT:        Priority Journals
ENTRY MONTH:         197702
ENTRY DATE:          Entered STN: 19900313
                     Last Updated on STN: 19900313
                     Entered Medline: 19770216
AB   Lambda-transducing phages carrying segments of the Escherichia coli
     chromosome in the aroE-trkA region have been isolated and shown by
     hybridization to carry an rRNA gene (rrnD).  The most likely gene order is
     trkA aroE rrnD.  The EcoRI and SmaI endonuclease cutting pattern of the
     rrnD gene is identical with the one of rrnB, differented from rrnC.

L12  ANSWER 2 OF 5  EMBASE  COPYRIGHT (c) 2005 Elsevier B.V. All rights
     reserved on STN
ACCESSION NUMBER:    78013410  EMBASE
DOCUMENT NUMBER:     1978013410
TITLE:               Coli phages found in human feces as free virions.
AUTHOR:              Kawabata N.; Hirakawa K.; Mukai H.; Nakamura K.
CORPORATE SOURCE:    Dept. Bacteriol., Fac. Med., Kagoshima Univ., Kagoshima,
                     Japan
SOURCE:              Acta Medica Universitatis Kagoshimaensis, (1976) Vol. 18,
                     No. 2, pp. 103-112.
```

CODEN: AMUKAC
DOCUMENT TYPE: Journal
FILE SEGMENT: 047 Virology
004 Microbiology
LANGUAGE: English
AB The present studies provided information on the density and properties of coliphages which existed as free virions in freshly excreted human feces. The phages active (propagating) on coli strains B and C and certain shigella strains were concerned. Marked differences were observed in phage content among individual fecal samples. Three of 9 samples contained coli phages in numbers sufficient to be detected by the plating method. The density was of the order of 103 to 104 PFU/ml irrespective of the host strains used. Coli cells in the same samples counting 105 to 106/ml, the phage/cell ratios ranged between 10-2 and 10-3. Seventy four phages were isolated and examined. They were classified into more than 5 types on the basis of their morphological and serological features. Forty one phages evidenced a morphological appearance characteristic to T even coli phages, and 26 among them were related serologically as well to T even phages. So it follows that true and related T even forms were widespread and predominant in human intestinal tract as free virions. There were also obtained 6 phages which resembled morphologically E1 phage of Bradley (1963) but were heterogenous in serological features, and, furthermore, 3 phages which were morphologically comparable to the **lamnda** phage but serologically distinct from it. The lambda type phages, though propagated on E. coli C, lysed none of the coli isolates from fecal samples.

L12 ANSWER 3 OF 5 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1994:622437 SCISEARCH
THE GENUINE ARTICLE: NF684
TITLE: M-FUNCTION BEHAVIOR FOR A PERIODIC DIRAC SYSTEM
AUTHOR: CLEMENCE D P (Reprint)
CORPORATE SOURCE: UNIV ZIMBABWE, DEPT MATH, POB MP 167, HARARE, ZIMBABWE (Reprint)
COUNTRY OF AUTHOR: ZIMBABWE
SOURCE: PROCEEDINGS OF THE ROYAL SOCIETY OF EDINBURGH SECTION A-MATHEMATICS, (1994) Vol. 124, Part 1, pp. 149-159. ISSN: 0308-2105.
PUBLISHER: ROYAL SOC EDINBURGH, 22-24 GEORGE ST, EDINBURGH, MIDLOTHIAN, SCOTLAND EH2 2PQ.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: PHYS
LANGUAGE: English
REFERENCE COUNT: 3
ENTRY DATE: Entered STN: 1994
Last Updated on STN: 1994
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB For a 2 x 2 periodic system with a perturbation P whose first moment is finite, Jy' = [lambdaI + R(x) + P(x)]y, we study the behaviour of the Titchmarsh-Weyl m(lambda)-coefficient at the spectral gap endpoints. Assuming gap nondegeneracy, our main results is that as lambda --> lambda0, (**lamnda** - lambda0)1/2 --> not-equal 0 if and only if lambda0 is a phi-half-bound state, which follows from an analysis of Jost-type functions.

L12 ANSWER 4 OF 5 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1991:655190 SCISEARCH
THE GENUINE ARTICLE: GR307
TITLE: PROJECTING PRECIPITOUSNESS
AUTHOR: GOLDRING N (Reprint)
CORPORATE SOURCE: UNIV CALIF LOS ANGELES, DEPT MATH, LOS ANGELES, CA 90024 (Reprint)

COUNTRY OF AUTHOR: USA
SOURCE: ISRAEL JOURNAL OF MATHEMATICS, (1991) Vol. 74, No. 1, pp. 13-31.
ISSN: 0021-2172.
PUBLISHER: MAGNES PRESS, PO BOX 7695, JERUSALEM 91076, ISRAEL.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: PHYS
LANGUAGE: English
REFERENCE COUNT: 10
ENTRY DATE: Entered STN: 1994
Last Updated on STN: 1994
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB This paper is about "strong" ideals on small cardinals. It is shown that a typical property of large cardinal measures does not transfer to these ideals. More specifically, that precipitous ideals on P-omega-1-lambda spaces may not project down to precipitous ideals on "smaller" P-omega-1-**lamnda**' spaces. Also, that the existence of a presaturated ideal on the bigger space does not imply the existence of a presaturated ideal on the smaller space.

L12 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2005:1161439 HCAPLUS
DOCUMENT NUMBER: 143:412054
TITLE: Measurement of Lambda(c) branching fractions of Cabibbo-suppressed decay modes in the BABAR experiment
AUTHOR(S): Saleem, M.
CORPORATE SOURCE: State Univ. of New York, Albany, NY, USA
SOURCE: (2005) 144 pp. Avail.: UMI, Order No. DA3164608
From: Diss. Abstr. Int., B 2005, 66(2), 961
DOCUMENT TYPE: Dissertation
LANGUAGE: English
AB Unavailable

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FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005

L1 42118 S STREPTOKINASE?
L2 630 S "SKC" OR "SKC-2"
L3 42539 S L1 OR L2
L4 7454183 S CLON? OR EXPRESS? OR RECOMBINANT
L5 4154 S L3 AND L4
L6 1362 S EQUISIMILIS
L7 220 S L5 AND L6
L8 49433 S INCLUSION (W) BOD?
L9 5 S L7 AND L8
L10 1 DUP REM L9 (4 DUPLICATES REMOVED)
L11 106 DUP REM L7 (114 DUPLICATES REMOVED)
L12 5 S LAMNDA

=> d l11 1-106 ibib ab

L11 ANSWER 1 OF 106 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN
ACCESSION NUMBER: 2005441717 EMBASE
TITLE: Heberkinasa: **Recombinant streptokinase** [9].
AUTHOR: Hernandez L.; Martinez Y.; Quintana M.; Besada V.; Martinez E.
CORPORATE SOURCE: L. Hernandez, Production Division, Centro de Ingenieria Genetica Y Biotecnologia, Ave 31 e/ 158 y 190, Cubanacan,

Playa, Habana 0600, Cuba. luciano.hernandez@cigb.edu.cu
SOURCE: European Heart Journal, (2005) Vol. 26, No. 16, pp. 1691. Refs: 4
ISSN: 0195-668X CODEN: EHJODF
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Letter
FILE SEGMENT: 018 Cardiovascular Diseases and Cardiovascular Surgery
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
ENTRY DATE: Entered STN: 20051020
Last Updated on STN: 20051020

L11 ANSWER 2 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:55585 SCISEARCH
THE GENUINE ARTICLE: 758JR
TITLE: Acquisition of host plasmin activity by the swine pathogen Streptococcus suis serotype 2
AUTHOR: Jobin M C; Brassard J; Quessy S; Gottschalk M; Grenier D (Reprint)
CORPORATE SOURCE: Univ Laval, Grp Rech Ecol Buccale, Fac Med Dent, Quebec City, PQ G1K 7P4, Canada (Reprint); Univ Montreal, Fac Med Vet, Grp Rech Malad Infect Porc, St Hyacinthe, PQ J2S 7C6, Canada; Univ Montreal, Fac Med Vet, Canadian Res Network Bacterial Pathogens Swine, Nat Sci & Engn Res Council Canada, St Hyacinthe, PQ J2S 7C6, Canada
COUNTRY OF AUTHOR: Canada
SOURCE: INFECTION AND IMMUNITY, (JAN 2004) Vol. 72, No. 1, pp. 606-610.
ISSN: 0019-9567.
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 28
ENTRY DATE: Entered STN: 23 Jan 2004
Last Updated on STN: 23 Jan 2004
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In this study, the plasminogen-binding activity of Streptococcus suis serotype 2 was investigated. Bound human plasminogen was activated by purified **streptokinase**, urokinase, or Streptococcus dysgalactiae subsp. **equisimilis** culture supernatant. Both human and porcine plasminogen were bound by S. suis. Binding was inhibited by E-aminocaproic acid, and the plasminogen receptor was heat and sodium dodecyl sulfate resistant. One of the receptors was identified as glyceraldehyde-3-phosphate dehydrogenase. S. suis-associated plasmin activity was capable of activating free plasminogen, which in turn could contribute to degradation of fibronectin. This is the first report on the plasminogen-binding activity of S. suis. Further studies may reveal a contribution of this activity to the virulence of S. suis.

L11 ANSWER 3 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:897332 HCAPLUS
DOCUMENT NUMBER: 142:190437
TITLE: Development of a green fluorescent protein metastatic-cancer chick-embryo drug-screen model
AUTHOR(S): Bobek, Vladimir; Plachy, Jiri; Pinterova, Daniela; Kolostova, Katarina; Boubelik, Michael; Jiang, Ping; Yang, Meng; Hoffman, Robert M.
CORPORATE SOURCE: Department of Molecular Biology, Third Faculty of Medicine Charles University Prague, Prague, Czech Rep.
SOURCE: Clinical & Experimental Metastasis (2004), 21(4), 347-352

CODEN: CEXMD2; ISSN: 0262-0898
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The chick-embryo model has been an important tool to study tumor growth, metastasis, and angiogenesis. However, an imageable model with a genetic fluorescent tag in the growing and spreading cancer cells that is stable over time has not been developed. We report here the development of such an imageable fluorescent chick-embryo metastatic cancer model with the use of green fluorescent protein (GFP). Lewis lung carcinoma cells, stably **expressing** GFP, were injected on the 12th day of incubation in the chick embryo. GFP-Lewis lung carcinoma metastases were visualized by fluorescence, after seven days addnl. incubation, in the brain, heart, and sternum of the developing chick embryo, with the most frequent site being the brain. The combination of **streptokinase** and gemcitabine was evaluated in this GFP metastatic model. Twelve-day-old chick embryos were injected i.v. with GFP-Lewis lung cancer cells, along with these two agents either alone or in combination. The **streptokinase** -gemcitabine combination inhibited metastases at all sites. The ED of gemcitabine was found to be 10 mg/kg and **streptokinase** 2000 IU per embryo. The data in this report suggest that this new stably fluorescent imageable metastatic-cancer chick-embryo model will enable rapid screening of new antimetastatic agents. Abbreviation: GFP - green fluorescent protein.
REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 4 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:69727 SCISEARCH
THE GENUINE ARTICLE: 763KK
TITLE: **Streptokinase** - a clinically useful thrombolytic agent
AUTHOR: Banerjee A; Chisti Y; Banerjee U C (Reprint)
CORPORATE SOURCE: Natl Inst Pharmaceut Educ & Res, Dept Biotechnol, Sector 67, Mohali 160062, Punjab, India (Reprint); Natl Inst Pharmaceut Educ & Res, Dept Biotechnol, Mohali 160062, Punjab, India; Massey Univ, Inst Technol & Engn, Palmerston North, New Zealand
COUNTRY OF AUTHOR: India; New Zealand
SOURCE: BIOTECHNOLOGY ADVANCES, (FEB 2004) Vol. 22, No. 4, pp. 287-307.
ISSN: 0734-9750.
PUBLISHER: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND.
DOCUMENT TYPE: General Review; Journal
LANGUAGE: English
REFERENCE COUNT: 171
ENTRY DATE: Entered STN: 30 Jan 2004
Last Updated on STN: 30 Jan 2004
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB A failure of hemostasis and consequent formation of blood clots in the circulatory system can produce severe outcomes such as stroke and myocardial infraction. Pathological development of blood clots requires clinical intervention with fibrinolytic agents such as urokinase, tissue plasminogen activator and **streptokinase**. This review deals with **streptokinase** as a clinically important and cost-effective plasminogen activator. The aspects discussed include: the mode of action; the structure and structure-function relationships; the structural modifications for improving functionality; **recombinant streptokinase**; microbial production; and recovery of this protein from crude broths. (C) 2003 Published by Elsevier Inc.

L11 ANSWER 5 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on

STN
ACCESSION NUMBER: 2004:656928 SCISEARCH
THE GENUINE ARTICLE: 837ZE
TITLE: Genetic analysis of Streptococcus uberis plasminogen activators
AUTHOR: Ward P N (Reprint); Leigh J A
CORPORATE SOURCE: Inst Anim Hlth, Compton Lab, Newbury RG20 7HN, Berks, England (Reprint); Inst Anim Hlth, Compton Lab, Compton RG20 7NN, Berks, England
phil.ward@bbscr.ac.uk
COUNTRY OF AUTHOR: England
SOURCE: INDIAN JOURNAL OF MEDICAL RESEARCH, (MAY 2004) Vol. 119, Supp. [S], pp. 136-140.
ISSN: 0971-5916.
PUBLISHER: INDIAN COUNCIL MEDICAL RES, PO BOX 4911 ANSARI NAGAR, NEW DELHI 110029, INDIA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 14
ENTRY DATE: Entered STN: 13 Aug 2004
Last Updated on STN: 13 Aug 2004
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background & objectives: Streptococci produce a diverse range of secreted plasminogen activators capable of converting mammalian plasminogen to plasmin in a species-specific manner. In all examples to date, the host animal's plasminogen and that of a number of additional species have been shown to interact with these molecules leading to the conclusion that the pathogenesis. of streptococci is in some way dependent upon activation of host plasminogen. PauA was the first plasminogen activator described from Streptococcus uberis, a pathogen frequently isolated from cases of bovine mastitis. Recently, a second S. uberis plasminogen activator (PauB) was identified from a Danish mastitis isolate. Interestingly, the pauB open reading frame occupied the locus normally filled by pauA. In the present study a genetic screen of streptococcal and field isolates frequently associated with mastitis was undertaken to assess the distribution, chromosomal location and sequence variation of these putative virulence factors.

Methods: Southern analysis of a diverse panel of streptococci and additional bacterial isolates frequently associated with bovine mastitis was performed using pauA and pauB probes. Sequence variation of PauA was assessed at the protein level following nucleotide sequence analysis of pauA alleles amplified from isolates picked from different geographical locations.

Results: We observed plasminogen activators to be universally distributed amongst S. uberis. A pauA allele was identified in all but one strain of S. uberis. This strain had a pauB allele substituted for pauA at the same locus. The remarkably low level of sequence variation demonstrated by PauA was further restricted to a limited number of residues within the molecule.

Interpretation & conclusion: The high prevalence of PauA alleles in, field isolates of S. uberis supported the observation that plasminogen activators are likely to confer an advantage with respect to colonization and growth. The findings of the present study support the theory that PauA plays a critical role in the pathogenesis of S. uberis.

L11 ANSWER 6 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 1
ACCESSION NUMBER: 2004:656911 SCISEARCH
THE GENUINE ARTICLE: 837ZE
TITLE: Control of **streptokinase** gene **expression** in group A & C streptococci by two-component regulators
AUTHOR: Malke H (Reprint); Steiner K
CORPORATE SOURCE: Univ Jena, Inst Mol Biol, Winzerlaer Str 10, D-07745 Jena, Germany (Reprint); Univ Jena, Inst Mol Biol, D-07745 Jena,

Germany
hmalke@imb-jena.de
COUNTRY OF AUTHOR: Germany
SOURCE: INDIAN JOURNAL OF MEDICAL RESEARCH, (MAY 2004) Vol. 119, Supp. [S], pp. 48-56.
ISSN: 0971-5916.
PUBLISHER: INDIAN COUNCIL MEDICAL RES, PO BOX 4911 ANSARI NAGAR, NEW DELHI 110029, INDIA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 23
ENTRY DATE: Entered STN: 13 Aug 2004
Last Updated on STN: 13 Aug 2004
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background & objectives: Group A streptococci (GAS) and human isolates of group C streptococci (GCS) have the stable capacity to produce the plasminogen activator **streptokinase**, albeit with varying efficiency. This property is subject to control by two two-component regulatory systems, FasCAX and CovRS, which act as activator and repressor, respectively. The present work aims at balancing these opposing activities in GAS and GCS, and at clarifying the phylogenetic position of the FasA response regulator, the less understood regulator of the two systems.

Methods: The GCS strain H46A and GAS strain NZ131 were used. Escherichia coli JM 109 was used as host for plasmid construction. **Streptokinase** activity of various wild type and mutant strains was measured. Phylogenetic trees of streptococcal FasA homologues were established.

Results: The **streptokinase** activities of the GAS strain NZ131 and the GCS strain H46A were attributable to more efficient CovR repressor action in NZ131 than in H46A. The FasA activator, on the other hand, functioned about equally efficient in the two strains. Phylogenetically, FasA homologues clustered distinctly in the proposed FasA-BlpR-ComE family of streptococcal response regulators and used the LytTR domain for DNA binding.

Interpretation & conclusion: Assessing the apparent **streptokinase** activity of streptoccal strains require the dissection of the activities of the cov and fas systems. Although experimental evidence is still missing, FasA is closely related to a widely distributed family of streptococcal response regulators that is involved in behavioral processes, such as quorum sensing.

L11 ANSWER 7 OF 106 EMBASE DUPLICATE 2
ACCESSION NUMBER: 2003404343 EMBASE
TITLE: **Expression** of streptodornase by use of **streptokinase** promoter in Streptococcus equismilis H46A.
AUTHOR: Sohn H.-J.; Chin J.; Kim I.-C.; Bai S.; Lee H.B.
CORPORATE SOURCE: H.B. Lee, Department of Biological Sciences, Chonnam National University, Gwangju 500-757, Korea, Republic of. blaise@chonnam.chonnam.ac.kr
SOURCE: Korean Journal of Microbiology and Biotechnology, (2003) Vol. 31, No. 3, pp. 307-310.
Refs: 18
ISSN: 1598-642X CODEN: HMHAAS
COUNTRY: Korea, Republic of
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: Korean
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20031023
Last Updated on STN: 20031023

AB A gene encoding streptodornase(sdc) from Streptococcus **equisimilis**

H46A was **expressed** in S. **equisimilis** H46A sdc(-) under the control of the **streptokinase** gene promoter. Secretion of the streptodornase was directed by the signal sequences of **streptokinase** or streptodornase. The **expressed** streptodornase activity from S. **equisimilis** H46A sdc(-) transformant with **streptokinase** promoter - streptodornase coding sequence fusion vector was 2.3 fold higher than that from wild type. Construct of signal sequence region replaced by **streptokinase** ones was similarly **expressed** as a wild type. But constructs of **skc** or lrp core regions of **streptokinase** promoter streptodornase fusion were similarly **expressed** as in sdc(-) mutant. In conclusion, improved **expression** of streptodornase by use of **streptokinase** promoter required the full length of promoter.

L11 ANSWER 8 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 3

ACCESSION NUMBER: 2003:133401 BIOSIS
DOCUMENT NUMBER: PREV200300133401
TITLE: Structural correlates of a functional **streptokinase** antigenic epitope: Serine 138 is an essential residue for antibody binding.
AUTHOR(S): Parhami-Seren, Behnaz [Reprint Author]; Seavey, Matthew; Krudysz, Jolanta; Tsantili, Panayota
CORPORATE SOURCE: Department of Biochemistry, College of Medicine, University of Vermont, 89 Beaumont Street, Given Building, Burlington, VT, 05405-0068, USA
bparhami@zoo.uvm.edu
SOURCE: Journal of Immunological Methods, (15 January 2003) Vol. 272, No. 1-2, pp. 93-105. print.
ISSN: 0022-1759 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 12 Mar 2003
Last Updated on STN: 12 Mar 2003

AB We determined the pattern of cross-reactivity of a panel of anti-**streptokinase** (SK) monoclonal antibodies (mAbs) with SK variants in order to map the antigenic and functional epitope of SK. Comparison of the pattern of cross-reactivity of the anti-SK mAb A4.3 with SK variants and sequence alignments of SK variants and native (n) SK suggested that mutation of Ser 138 to Lys results in loss of binding of mAb A4.3 to SK variants. However, this mutation does not affect formation of activator complex by these proteins. The epitope specificity of the mAb A4.3 was further confirmed by mutating Ser 138 to Lys in n SK. Monoclonal Ab A4.3 did not bind to mutant SK (Ser138Lys). Activator activity of mutant SK (Ser138Lys) was indistinguishable from that of n SK and **recombinant** n SK. Since addition of A4.3 mAb to an equimolar mixture of SK and human plasminogen inhibits activator complex formation, the sequences spanning position 138 are likely important for formation of **streptokinase**-plasminogen activator complex or processing of the plasminogen substrate.

L11 ANSWER 9 OF 106 MEDLINE on STN DUPLICATE 4

ACCESSION NUMBER: 2002322701 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12065504
TITLE: Dual control of **streptokinase** and streptolysin S production by the covRS and fasCAX two-component regulators in Streptococcus dysgalactiae subsp. **equisimilis**.
AUTHOR: Steiner Kerstin; Malke Horst
CORPORATE SOURCE: Institute for Molecular Biology, Friedrich Schiller University Jena, D-07745 Jena, Germany.
SOURCE: Infection and immunity, (2002 Jul) 70 (7) 3627-36.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AY075106; GENBANK-AY075107
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020615
Last Updated on STN: 20020731
Entered Medline: 20020730

AB Synthesis of the plasminogen activator **streptokinase** (SK) by group A streptococci (GAS) has recently been shown to be subject to control by two two-component regulators, covRS (or csrRS) and fasBCA. In independent studies, response regulator CovR proved to act as the repressor, whereas FasA was found to act indirectly as the activator by controlling the **expression** of a stimulatory RNA, fasX. In an attempt at understanding the regulation of SK production in the human group C streptococcal (GCS) strain H46A, the strongest SK producer known yet, we provide here physical and functional evidence for the presence of the cov and fas systems in GCS as well and, using a mutational approach, compare the balance between their opposing actions in H46A and GAS strain NZ131. Sequence analysis combined with Southern hybridization revealed that the covRS and fasCAX operons are preserved at high levels of primary structure identity between the corresponding GAS and GCS genes, with the exception of fasB, encoding a second sensor kinase that is not a member of the GCS fas operon. This analysis also showed that wild-type H46A is actually a derepressed mutant for SK and streptolysin S (SLS) synthesis, carrying a K102 amber mutation in covR. Using cov and fas mutations in various combinations together with strain constructs allowing complementation in trans, we found that, in H46A, cov and fas contribute to approximately equal negative and positive extents, respectively, to constitutive SK and SLS activity. The amounts of SK paralleled the level of **ska**(H46A) transcription. The most profound difference between H46A and NZ131 regarding the relative activities of the cov and fas systems consisted in significantly higher activity of a functional CovR repressor in NZ131 than in H46A. In NZ131, CovR decreased SK activity in a Fas(+) background about sevenfold, compared to a 1.9-fold reduction of SK activity in H46A. Combined with the very short-lived nature of covR mRNA (decay rate, 1.39/min), such differences may contribute to strain-specific peculiarities of the **expression** of two prominent streptococcal virulence factors in response to environmental changes.

L11 ANSWER 10 OF 106 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2002053807 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11779212
TITLE: Specificity role of the **streptokinase** C-terminal domain in plasminogen activation.
AUTHOR: Kim Dong Min; Lee Sang Jun; Yoon Suk Kwon; Byun Si Myung
CORPORATE SOURCE: Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), 305-701 Taejon, South Korea.
SOURCE: Biochemical and biophysical research communications, (2002 Jan 11) 290 (1) 585-8.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20020125
Last Updated on STN: 20020226
Entered Medline: 20020225

AB Several pathogenic bacteria secrete plasminogen activator proteins. **Streptokinase** (SKe) produced by Streptococcus **equisimilis** and staphylokinase secreted from Staphylococcus aureus are human

plasminogen activators and **streptokinase** (SKu), produced by Streptococcus uberis, is a bovine plasminogen activator. Thus, the fusion proteins among these activators can explain the function of each domain of SKe. Replacement of the SKalpha domain with staphylokinase donated the staphylokinase-like activation activity to SKe, and the SKbetagamma domain played a role of nonproteolytic activation of plasminogen. **Recombinant** SKu also activated human plasminogen by staphylokinase-like activation mode. Because SKu has homology with SKe, the bovine plasminogen activation activities of SKe fragments were checked. SKebetagamma among them had activation activity with bovine plasminogen. This means that the C-terminal domain (gamma-domain) of **streptokinase** determines plasminogen species necessary for activation and converses the ability of substrate recognition to human species.

L11 ANSWER 11 OF 106 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2002110457 EMBASE
TITLE: Can imperfections help to improve bioreactor performance?.
AUTHOR: Patnaik P.R.
CORPORATE SOURCE: P.R. Patnaik, Institute of Microbial Technology, Sector 39-A, Chandigarh-160 036, India. pratap@imtech.res.in
SOURCE: Trends in Biotechnology, (1 Apr 2002) Vol. 20, No. 4, pp. 135-137.
Refs: 22
ISSN: 0167-7799 CODEN: TRBIDM
PUBLISHER IDENT.: S 0167-7799(01)01922-9
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
027 Biophysics, Bioengineering and Medical Instrumentation
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20020404
Last Updated on STN: 20020404

AB Pilot-scale and larger bioreactors differ from small laboratory-scale reactors in terms of a greater occurrence of noise and incomplete mixing of the broth. Conventional control tries to induce good mixing and to filter out the noise as completely as possible. As such an 'ideal' operation is difficult to achieve, recent work has tried to exploit the non-ideal features to improve the performance. Using artificial neural networks, the degree of mixing, the extent of filtering of noise and the distribution of plasmid copy number (in a **recombinant** fermentation) can be controlled effectively on-line. This strategy generates better productivities than well-mixed noise-free operations, which suggests that deviations from ideal behaviour should be gainfully harnessed and not suppressed.

L11 ANSWER 12 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2002:21007 SCISEARCH
THE GENUINE ARTICLE: 503LT
TITLE: Characterization of PauB, a novel broad-spectrum plasminogen activator from Streptococcus uberis
AUTHOR: Ward P N; Leigh J A (Reprint)
CORPORATE SOURCE: Compton Lab, Inst Anim Hlth, Compton RG20 7NN, Berks, England (Reprint)
COUNTRY OF AUTHOR: England
SOURCE: JOURNAL OF BACTERIOLOGY, (JAN 2002) Vol. 184, No. 1, pp. 119-125.
ISSN: 0021-9193.
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC

20036-2904 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 35
ENTRY DATE: Entered STN: 11 Jan 2002
Last Updated on STN: 11 Jan 2002
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A bovine plasminogen activator of atypical molecular mass (similar to 45 kDa) from Streptococcus uberis strain SK880 had been identified previously (L. B. Johnsen, K. Poulsen, M. Kilian, and T. E. Petersen. Infect. Immun. 67:1072-1078, 1999). The strain was isolated from a clinical case of bovine mastitis. The isolate was found not to secrete PauA, a bovine plasminogen activator **expressed** by the majority of S. uberis strains. Analysis of the locus normally occupied by pauA revealed an absence of the pauA open reading frame. However, an alternative open reading frame was identified within the same locus. Sequence analysis of the putative gene suggested limited but significant homology to other plasminogen activators. A candidate signal peptide sequence and cleavage site were also identified. **Expression cloning** of DNA encoding the predicted mature protein (lacking signal peptide) confirmed that the open reading frame encoded a plasminogen activator of the expected size, which we have named PauB. Both native and **recombinant** forms of PauB displayed an unexpectedly broad specificity profile for bovine, ovine, equine, caprine, porcine, rabbit, and human plasminogen. Clinical and nonclinical field isolates from nine United Kingdom sites were screened for the pauB gene and none were identified as carrying it. Similarly, clinical isolates from 20 Danish herds were all found to encode PauA and not PauB. Therefore, PauB represents a novel but rare bacterial plasminogen activator which displays very broad specificity.

L11 ANSWER 13 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:471828 HCAPLUS
DOCUMENT NUMBER: 133:99568
TITLE: **Streptokinase** derivatives with high affinity for activated platelets and methods of their production and use in thrombolytic therapy
INVENTOR(S): Galler, Lawrence Isaac
PATENT ASSIGNEE(S): USA
SOURCE: U.S., 15 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6087332	A	20000711	US 1997-997532	19971223

PRIORITY APPLN. INFO.: US 1997-997532 19971223
OTHER SOURCE(S): MARPAT 133:99568

AB **Streptokinase** derivs. having platelet glycoprotein-binding domains adjacent to the termini of the **streptokinase** sequence are disclosed. These derivs. produce higher local concns. of plasmin in vivo as compared to unmodified **streptokinase**. Certain of the derivs. have high affinity for the GPIIB/IIIA receptor and low affinity for the fibronectin and vitronectin receptors. Others have substantially equivalent affinity for all three receptors. The derivs. are useful in treating thromboembolic disorders. The **streptokinase** derivs. can be made by **recombinant** techniques or by chemical synthesis or conjugation.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 14 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2005:29440 HCAPLUS
DOCUMENT NUMBER: 142:428913
TITLE: An improved process for the simultaneous preparation of extracellular **streptokinase** and its analogues
INVENTOR(S): Dikshit, Kanak Lata; Vyas, Vinay Venkatrao; Mahajan, Ritu; Kaur, Jaodeep; Thapar, Nitika; Phatap, Jitesh; Nihalani, Deepak; Sahni, Girish
PATENT ASSIGNEE(S): Council of Scientific and Industrial Research, India
SOURCE: Indian, 122 pp.
CODEN: INXXAP
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
IN 183828	A	20000429	IN 1994-DE1727	19941230
PRIORITY APPLN. INFO.:			IN 1994-DE1727	19941230

AB Extracellular **streptokinase** and its analogs prepared by growing **recombinant** E.coli in a conventional fermentation medium under stirring and supplemented with aeration, separating the cells from supernatant by known methods followed by recovering and purifying **Streptokinase** and its analogs from supernatant.

L11 ANSWER 15 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:175570 HCAPLUS
DOCUMENT NUMBER: 132:218864
TITLE: **Streptokinase** analogs with low antigenicity for use as thrombolytics
INVENTOR(S): Torrens Madrazo, Isis Del Carmen; Garcia Ojalvo, Ariana; De La Fuente Garcia, Jose De Jesus; Seralena Menendez, Alina
PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba
SOURCE: Eur. Pat. Appl., 54 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 985729	A2	20000315	EP 1999-202639	19990813
EP 985729	A3	20000531		
EP 985729	B1	20050427		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
AU 9943424	A1	20000309	AU 1999-43424	19990805
AU 769916	B2	20040212		
CA 2277554	C	20041102	CA 1999-2277554	19990806
CA 2277554	AA	20000214		
US 6309873	B1	20011030	US 1999-374038	19990813
AT 294239	E	20050515	AT 1999-202639	19990813
US 6413759	B1	20020702	US 2000-658179	20000908
PRIORITY APPLN. INFO.:			CU 1998-119 A	19980814
			US 1999-374038 A3	19990813

AB **Streptokinase** analogs with antigenic domains modified to minimize antigenicity are described for use in the treatment of clotting-associated disorders. The proteins retain their capacity for plasminogen activator complex formation. The proteins are manufactured by

expression of the corresponding allele of the skc2 gene encoding **streptokinase** SKC-2 (Heberkinase®). The mols. obtained from present invention can be used in the treatment of disorders as myocardial infarction, pulmonary thromboembolism, surgical complications and other cases of thrombosis.

L11 ANSWER 16 OF 106 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2001003323 EMBASE
TITLE: Leucine 42 in the fibronectin motif of **streptokinase** plays a critical role in fibrin-independent plasminogen activation.
AUTHOR: Liu L.; Sazonova I.Y.; Turner R.B.; Chowdhry S.A.; Tsai J.; Houng A.K.; Reed G.L.
CORPORATE SOURCE: G.L. Reed, Cardiovascular Biology Laboratory, HSPH II-127, 677 Huntington Ave., Boston, MA 02115, United States. reed@cvlab.harvard.edu
SOURCE: Journal of Biological Chemistry, (1 Dec 2000) Vol. 275, No. 48, pp. 37686-37691.
Refs: 35
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20010111
Last Updated on STN: 20010111

AB The NH(2) terminus (residues 1-59) of **streptokinase** (SK) is a molecular switch that permits fibrin-independent plasminogen activation. Targeted mutations were made in **recombinant** (r) SK1-59 to identify structural interactions required for this process. Mutagenesis established the functional roles of Phe-37and Glu-39, which were projected to interact with microplasmin in the activator complex. Mutation of Leu-42 (rSK1-59(L42A)), a conserved residue in the SK fibronectin motif that lacks interactions with microplasmin, strongly reduced plasminogen activation (k(cat) decreased 50-fold) but not arnidolysis (k(cat) decreased 1.5-fold). Otherwise rSK1-59(L42A) and native rSK1-59 were indistinguishable in several parameters. Both displayed saturable and specific binding to Glu-plasminogen or the remaining SK fragment (rSKA59). Similarly rSK1-59 and rSK1-59(L42A) bound simultaneously to two different plasminogen molecules, indicating that both plasminogen binding sites were intact. However, when bound to SKΔ59, rSK1-59(L42A) was less effective than rSK1-59 in restructuring the native conformation of the SK A domain, as detected by conformation-dependent monoclonal antibodies. In the light of previous studies, these data provide evidence that SK1-59 contributes to fibrin-independent plasminogen activation through 1) intermolecular inter-actions with the plasmin in the activator complex, 2) binding interactions with the plasminogen substrate, and 3) intramolecular interactions that structure the A domain of SK for Pg substrate processing.

L11 ANSWER 17 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:148580 SCISEARCH
THE GENUINE ARTICLE: 285UW
TITLE: Allele substitution of the **streptokinase** gene reduces the nephritogenic capacity of group A streptococcal strain NZ131
AUTHOR: Nordstrand A (Reprint); McShan W M; Ferretti J J; Holm S E; Norgren M
CORPORATE SOURCE: Umea Univ, Dept Clin Bacteriol, S-90185 Umea, Sweden (Reprint); Univ Oklahoma, Hlth Sci Ctr, Dept Microbiol & Immunol, Oklahoma City, OK 73190 USA

COUNTRY OF AUTHOR: Sweden; USA
SOURCE: INFECTION AND IMMUNITY, (MAR 2000) Vol. 68, No. 3, pp. 1019-1025.
ISSN: 0019-9567.
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 36
ENTRY DATE: Entered STN: 2000
Last Updated on STN: 2000
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB To investigate the role of allelic variants of **streptokinase** in the pathogenesis of acute poststreptococcal glomerulonephritis (APSGN), site-specific integration plasmids were constructed, which contained either the non-nephritis-associated **streptokinase** gene (skc5) from the group C streptococcal strain Streptococcus **equisimilis** H46A or the nephritis-associated **streptokinase** gene (ska1) from the group A streptococcal nephritogenic strain NZ131. The plasmids were introduced by electroporation and homologous recombination into the chromosome of an isogenic derivative of strain NZ131, in which the **streptokinase** gene had been deleted and which had thereby lost its nephritogenic capacity in a mouse model of APSGN. The introduction of a non-nephritis-associated allelic variant of **streptokinase** did not rescue the nephritogenic capacity of the strain. The mutant and the wild-type strains produced equivalent amounts of **streptokinase**. Complementation of the ska deletion derivative with the original ska allele reconstituted the nephritogenicity of wild-type NZ131. The findings support the hypothesis that the role of **streptokinase** in the pathogenesis of APSGN is related to the allelic variant of the protein.

L11 ANSWER 18 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:100732 SCISEARCH
THE GENUINE ARTICLE: 277JH
TITLE: Genetic organisation of the M protein region in human isolates of group C and G streptococci: two types of multigene regulator-like (mgrC) regions
AUTHOR: Geyer A; Schmidt K H (Reprint)
CORPORATE SOURCE: Univ Jena, Univ Hosp, Inst Med Microbiol, Semmelweissstr 4, D-07740 Jena, Germany (Reprint); Univ Jena, Univ Hosp, Inst Med Microbiol, D-07740 Jena, Germany
COUNTRY OF AUTHOR: Germany
SOURCE: MOLECULAR AND GENERAL GENETICS, (JAN 2000) Vol. 262, No. 6, pp. 965-976.
ISSN: 0026-8925.
PUBLISHER: SPRINGER-VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 47
ENTRY DATE: Entered STN: 2000
Last Updated on STN: 2000
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In addition to beta-haemolytic streptococci belonging to Lancefield group A (Streptococcus pyogenes, GAS), human isolates of group C (GCS) and group G (GGS) streptococci (S. dysgalactiae subsp. **equisimilis**:) have been implicated as causative agents in outbreaks of purulent pharyngitis, of wound infections and recently also of streptococcal toxic shock-like syndrome. Very little is known about the organisation of the genomic region in which the emm gene of GCS and GCS is located. We have investigated the genome sequences flanking the emm gene in GCS by sequencing neighbouring fragments obtained by inverse PCR. Our sequence data for GCS strains 25287 and H46A revealed two types of arrangement in

the emm region, which differ significantly from the known types of mga regulon in GAS. We named this segment of the genome mgrC (for multigene regulon-like segment in group C streptococci). In strains belonging to the first mgrC type (prototype strain 25287) the emm gene is flanked upstream by mgc, a gene that is 61% identical to the mga gene of GAS. A phylogenetic analysis of the deduced protein sequences showed that Mgc is related to Mga proteins of various types of GAS but forms a distinct cluster. Downstream of emm, the mgrC sequence region is bordered by rel. This gene encodes a protein that functions in the synthesis and degradation of guanosine 3',5' bipyrophosphate (ppGpp) during the stringent regulatory response to amino acid deprivation. In the second mgrC type (prototype strain H46A), the genes mgc and emm are arranged as in type 1. But an additional ORF (orf) is inserted in opposite orientation between emm and rel. This orf shows sequence homology to cpdB, which is present in various microorganisms and encodes 2',3' cyclo-nucleotide 2'-phosphodiesterase. PCR analysis showed that these two mgrC arrangements also exist in GGS. Our sequence and PCR data further showed that both types of mgrC region in GCS and GGS are linked via rel to the **streptokinase** region characterised recently in strain H46A.
A gene encoding C5a peptidase, which is present at the 3' end of the mga regulon in GAS, was not found in the mgrC region identified in the GCS and GGS strains investigated here.

L11 ANSWER 19 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:126480 HCAPLUS
DOCUMENT NUMBER: 133:218185
TITLE: Firefly luciferase as a reporter to study gene **expression** in Streptococcus mutans. [Erratum to document cited in CA131:347148]
AUTHOR(S): Goodman, Steven D.; Gao, Qian
CORPORATE SOURCE: Department of Basic Sciences, University of Southern California School of Dentistry, Los Angeles, CA, 90089-0641, USA
SOURCE: Plasmid (2000), 43(2), 184
CODEN: PLSMDX; ISSN: 0147-619X
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The firefly (Photinus pyralis) luciferase coding sequence has previously been used as a reporter in the genus Streptococcus [S. Grafe, T. Ellinger, and H. Malke (1996) Structural dissection and functional anal. of the complex promoter of the **streptokinase** gene from Streptococcus **equisimilis** H46A. Med. Microbiol. Immunol. 185, 11-17]. The text on page 154, second paragraph, lines 13 and 14 should be amended to read "...it has been underutilized in gram-pos. hosts.". (c) 2000 Academic Press.

L11 ANSWER 20 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 6
ACCESSION NUMBER: 2000:387699 BIOSIS
DOCUMENT NUMBER: PREV200000387699
TITLE: **Expression** and regulation of the **streptokinase** gene.
AUTHOR(S): Malke, Horst [Reprint author]; Steiner, Kerstin; Gase, Klaus; Frank, Carsten
CORPORATE SOURCE: Institute for Molecular Biology, Friedrich Schiller University Jena, D-07745, Jena, Germany
SOURCE: Methods (Orlando), (June, 2000) Vol. 21, No. 2, pp. 111-124. print.
CODEN: MTHDE9. ISSN: 1046-2023.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 13 Sep 2000
Last Updated on STN: 8 Jan 2002

AB Recent research in various areas has appreciably expanded our knowledge of **streptokinase**, a plasminogen activator produced by all human group A (GAS), group C (GCS), and group G (GGS) streptococci. Several molecular genetic approaches are described here to study the **expression** of the **streptokinase** gene, skn. Southern hybridization analysis demonstrated homology of synteny of ska, **skc**, and skg in the genomes of the above serogroups. S1 nuclease mapping, the use of transcriptional fusions to BETA-galactosidase and luciferase reporter genes, in conjunction with site-directed mutagenesis, led to the localization of the core promoter region of **skc** and the identification of a cis-active upstream region required for full promoter activity. Circular permutation analysis of the promoter upstream region identified an intrinsic DNA bending locus as the pivotal DNA element stimulating the activity of the core promoter. The detection of skn allele-specific **expression** phenotypes, which proved not to be due to different skn mRNA half-lives, prompted allele swap experiments, showing that promoter activity is dictated by the host genetic background, rather than the sequence of the regulatory region. These findings suggest the involvement in skn **expression** of an as yet unidentified transcriptional activator that contacts the bent DNA region. Transcription termination of **skc** is directed by a bidirectional terminator whose structural requirements for termination efficiency were determined with base substitution mutants fused to a chloramphenicol acetyl transferase reporter. Finally, mutagenic plasmids are described for insertion-duplication and allele replacement mutagenesis of the skn locus.

L11 ANSWER 21 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1999:405093 HCAPLUS
DOCUMENT NUMBER: 131:54027
TITLE: Fibrin-dependent plasminogen activator activity of modified bacterial **streptokinases**
INVENTOR(S): Reed, Guy L.
PATENT ASSIGNEE(S): The President and Fellows of Harvard College, USA
SOURCE: PCT Int. Appl., 73 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 9931247	A1	19990624	WO 1998-US26694		19981215
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM					
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG					
AU 9918295	A1	19990705	AU 1999-18295		19981215
US 6210667	B1	20010403	US 1998-211542		19981215
PRIORITY APPLN. INFO.:			US 1997-69497P	P	19971215
			WO 1998-US26694	W	19981215

AB A pharmaceutical composition in a preferred embodiment comprises an isolated bacterial protein **streptokinase** that induces fibrin-dependent plasminogen activation, and methods for dissolving blood clots in a subject using such a composition Two preferred **streptokinase** mutants and truncated derivs. comprising residues 144-293 and residues 60-414 of the Streptococcus **equisimilis** H46A enzyme. Deletion of the first 59 amino acids to product mutant rSK60-414 yielded a protein with a 767-fold decrease in kcat compared to that of rSK1-414, without any

significant change in the Km. The N-terminus dets. the clot (fibrin) dependence of plasminogen activation by **streptokinase** and the regulation of plasminogen activation in the presence of fibrin. By virtue of its requirement for fibrin for plasminogen activation in human plasma, and its sparing of fibrinogen during clot dissoln., **streptokinase** deleted of N-terminal amino acid residues is similar to tissue-type plasminogen activator. Embodiments also include a nucleic acid encoding such as a bacterial protein, a nucleic acid encoding such a bacterial protein as a fusion to another protein, an **expression** vector with the nucleic acid, and a host cell transformed with the **expression** vector.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 22 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1999:579938 HCAPLUS
DOCUMENT NUMBER: 131:183948
TITLE: Manufacture of **streptokinase** for therapeutic use by **expression** of the **cloned** gene in Pichia pastoris
INVENTOR(S): Estrada Garcia, Mario; Rubiera Chaplen, Roger; Perez, Hidalgo; Serrano Doce, Ricardo; Hernandez Marrero, Luciano F.; Rodriguez Collazo, Pedro; Castro Ramirez, Anaisel; Munoz Munoz, Emilio Amable; Bravo Martinez, Walfrido; Campos Somavilla, Magalys; Pedraza Fernandez, Alicia; De la Furente Garcia, Jose de J.; Herrera Martinez, Luis S.
PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba
SOURCE: Czech Rep., 18 pp.
CODEN: CZXXED
DOCUMENT TYPE: Patent
LANGUAGE: Czech
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
CZ 284692	B6	19990217	CZ 1991-2256		19910719
RU 2107726	C1	19980327	RU 1991-5001280		19910717
PRIORITY APPLN. INFO.:			CU 1990-90	A	19900523
			CS 1991-2256	A	19910719
			SU 1991-5001280	A	19910717

AB **Streptokinase** for therapeutic use is manufactured by **expression** of the **cloned** gene in Pichia pastoris. The protein may be secreted into the culture medium or accumulated intracellularly. The gene was **cloned** from a type C Streptococcus **equisimilis** by PCR. **Expression** of the gene from the AOX1 promoter using Pichia pastoris as the host resulted in the manufacture of an enzyme with a specific activity of 50,000-100,000 fibrin-agarose units/mg protein.

L11 ANSWER 23 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:88244 BIOSIS
DOCUMENT NUMBER: PREV200000088244
TITLE: Hydrodynamic radii of native and denatured proteins measured by pulse field gradient NMR techniques.
AUTHOR(S): Wilkins, Deborah K.; Grimshaw, Shaun B.; Receveur, Veronique; Dobson, Christopher M.; Jones, Jonathan A.; Smith, Lorna J. [Reprint author]
CORPORATE SOURCE: Oxford Centre for Molecular Sciences, New Chemistry Laboratory, University of Oxford, South Parks Road, Oxford, OX1 3QT, UK

SOURCE: Biochemistry, (Dec. 14, 1999) Vol. 38, No. 50, pp. 16424-16431. print.
CODEN: BICHAW. ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Mar 2000
Last Updated on STN: 3 Jan 2002

AB Pulse field gradient NMR methods have been used to determine the effective hydrodynamic radii of a range of native and nonnative protein conformations. From these experimental data, empirical relationships between the measured hydrodynamic radius (Rh) and the number of residues in the polypeptide chain (N) have been established; for native folded proteins Rh = 4.75N 0.29ANG and for highly denatured states Rh = 2.21N 0.57ANG. Predictions from these equations agree well with experimental data from dynamic light scattering and small-angle X-ray or neutron scattering studies reported in the literature for proteins ranging in size from 58 to 760 amino acid residues. The predicted values of the hydrodynamic radii provide a framework that can be used to analyze the conformational properties of a range of nonnative states of proteins. Several examples are given here to illustrate this approach including data for partially structured molten globule states and for proteins that are unfolded but biologically active under physiological conditions. These reveal evidence for significant coupling between local and global features of the conformational ensembles adopted in such states. In particular, the effective dimensions of the polypeptide chain are found to depend significantly on the level of persistence of regions of secondary structure or features such as hydrophobic clusters within a conformational ensemble.

L11 ANSWER 24 OF 106 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 2000038313 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10569766
TITLE: **Cloning, expression**, sequence analysis, and characterization of **streptokinases** secreted by porcine and equine isolates of Streptococcus **equisimilis**.
AUTHOR: Caballero A R; Lottenberg R; Johnston K H
CORPORATE SOURCE: Department of Microbiology, Immunology and Parasitology, Louisiana State University Medical Center, New Orleans, Louisiana 70112, USA.
CONTRACT NUMBER: R01DK45014 (NIDDK)
SOURCE: Infection and immunity, (1999 Dec) 67 (12) 6478-86.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF104300; GENBANK-AF104301
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991220

AB **Streptokinases** secreted by nonhuman isolates of group C streptococci (Streptococcus equi, S. **equisimilis**, and S. zooepidemicus) have been shown to bind to different mammalian plasminogens but exhibit preferential plasminogen activity. The **streptokinase** genes from S. **equisimilis** strains which activated either equine or porcine plasminogen were **cloned**, sequenced, and **expressed** in Escherichia coli. The **streptokinase** secreted by the equine isolate had little similarity to any known **streptokinases** secreted by either human or porcine isolates. The **streptokinase** secreted by the porcine isolate had limited structural and functional similarities to **streptokinases** secreted by human isolates. Plasminogen activation studies with

immobilized (His)(6)-tagged **recombinant streptokinases** indicated that these **recombinant streptokinases** interacted with plasminogen in a manner similar to that observed when **streptokinase** and plasminogen interact in the fluid phase. Analysis of the cleavage products of the **streptokinase** -plasminogen interaction indicated that human, equine, and porcine plasminogens were all cleaved at the same highly conserved site. The site at which **streptokinase** was cleaved to form altered **streptokinase** (Sk*) was also determined. This study confirmed not only the presence of **streptokinases** in nonhuman S. **equisimilis** isolates but also that these proteins belong to a family of plasminogen activators more diverse than previously thought.

L11 ANSWER 25 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 8

ACCESSION NUMBER: 2000:96119 BIOSIS
DOCUMENT NUMBER: PREV200000096119
TITLE: Two **streptokinase** genes are **expressed** with different solubility in Escherichia coli W3110.
AUTHOR(S): Pupo, Elder [Reprint author]; Baghbaderani, Behnam A.; Lugo, Victoria; Fernandez, Julio; Paez, Rolando; Torrens, Isis
CORPORATE SOURCE: Biopharmaceutical Development Division, Center for Genetic Engineering and Biotechnology, Havana, Cuba
SOURCE: Biotechnology Letters, (Dec., 1999) Vol. 21, No. 12, pp. 1119-1123. print.
CODEN: BILED3. ISSN: 0141-5492.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 15 Mar 2000
Last Updated on STN: 3 Jan 2002

AB The **streptokinase** (SK) gene from S. **equisimilis** H46A (ATCC 12449) was **cloned** in E. coli W3110 under the control of the tryptophan promoter. The **recombinant** SK, which represented 15% of total cell protein content, was found in the soluble fraction of disrupted cells. The solubility of this SK notably differed from that of the product of the SK gene from S. **equisimilis** (ATCC 9542) which had been **cloned** in E. coli W3110 by using similar **expression** vector and cell growth conditions, and occurred in the form of inclusion bodies.

L11 ANSWER 26 OF 106 MEDLINE on STN DUPLICATE 9

ACCESSION NUMBER: 1999150235 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10024545
TITLE: Purification and **cloning** of a **streptokinase** from Streptococcus uberis.
AUTHOR: Johnsen L B; Poulsen K; Kilian M; Petersen T E
CORPORATE SOURCE: Protein Chemistry Laboratory, Department of Molecular and Structural Biology, University of Aarhus, DK-8000 Aarhus C, Denmark.
SOURCE: Infection and immunity, (1999 Mar) 67 (3) 1072-8.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ131604; GENBANK-AJ131605; GENBANK-AJ131631
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990326
Last Updated on STN: 19990326
Entered Medline: 19990312

AB A bovine plasminogen activator was purified from the culture supernatant of the bovine pathogen Streptococcus uberis NCTC 3858. After the final reverse-phase high-performance liquid chromatography step a single protein

with a molecular mass of 32 kDa was detected in the active fraction. A partial peptide map was established, and degenerate primers were designed and used for amplification of fragments of the gene encoding the activator. Inverse PCR was subsequently used for obtaining the full-length gene. The S. uberis plasminogen activator gene (**skc**) encodes a protein consisting of 286 amino acids including a signal peptide of 25 amino acids. In an amino acid sequence comparison the **cloned** activator showed an identity of approximately 26% to the **streptokinases** isolated from Streptococcus **equisimilis** and Streptococcus pyogenes. Interestingly, the activator from S. uberis was found to lack the C-terminal domain possessed by the **streptokinase** from S. **equisimilis**. This is apparently a general feature of the **streptokinases** of this species; biochemical and genetic analysis of 10 additional strains of S. uberis revealed that 9 of these were highly similar to strain NCTC 3858. Sequencing of the **skc** gene from three of these strains indicated that the amino acid sequence of the protein is highly conserved within the species.

L11 ANSWER 27 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1999-03949 BIOTECHDS
TITLE: Purification and **cloning** of a **streptokinase** from Streptococcus uberis; cattle plasminogen-activator purification and characterization
AUTHOR: Johnson L B; Poulsen K; Kilian M; *Petersen T E
CORPORATE SOURCE: Univ.Aarhus
LOCATION: Protein Chemistry Laboratory, Gustav Wieds Vej 10C, DK-8000 Aarhus C, Denmark.
Email: tep@mbio.aau.dk
SOURCE: Infect.Immun.; (1999) 67, 3, 1072-78
CODEN: INFIBR
ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A cattle plasminogen-activator was purified from the culture supernatant of Streptococcus uberis NTCTC 3858. After the final reverse-phase HPLC step, a single protein with a mol.weight of 32,000 was detected in the active fraction. A partial peptide map was established, and degenerate DNA primers were designed and used for amplification of fragments of the gene encoding the activator. Inverse polymerase chain reaction was used for obtaining the full-length gene. The S. uberis plasminogen-activator gene (**skc**) encodes a protein consisting of 286 amino acids including a signal peptide of 25 amino acids. In a protein sequence comparison, the **cloned** activator showed an identity of approximately 26% to the **streptokinases** isolated from Streptococcus **equisimilis** and Streptococcus pyogenes. The activator from S. uberis lacked the C-terminal domain possessed by the **streptokinase** from S. **equisimilis**. This is apparently a general feature of the **streptokinases** of this species. Sequencing of the **skc** gene from 3 of these strains indicated that the protein sequence of the protein is highly conserved within the species. (32 ref)

L11 ANSWER 28 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1999:666643 SCISEARCH
THE GENUINE ARTICLE: 231GM
TITLE: PauA: a novel plasminogen activator from Streptococcus uberis
AUTHOR: Rosey E L; Lincoln R A; Ward P N; Yancey R J; Leigh J A (Reprint)
CORPORATE SOURCE: Inst Anim Hlth, Compton Lab, Newbury RG20 7NN, Berks, England (Reprint); Pfizer Inc, Div Cent Res, Anim Hlth

Biol Discovery, Groton, CT 06340 USA
COUNTRY OF AUTHOR: England; USA
SOURCE: FEMS MICROBIOLOGY LETTERS, (1 SEP 1999) Vol. 178, No. 1, pp. 27-33.
ISSN: 0378-1097.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 20
ENTRY DATE: Entered STN: 1999
Last Updated on STN: 1999
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Chromosomal DNA from two geographically distinct isolates of Streptococcus uberis was used to **clone** the plasminogen activator in an active form in Escherichia coli. The **cloned** fragments from each strain contained four potential open reading frames (ORFs). That for the plasminogen activator encoded a protein of 286 amino acids (33.4 kDa) which is cleaved between residues 25 and 26 during secretion by S. uberis. The amino acid sequence of the mature protein showed only weak homology (23.5-28%) to **streptokinase**. The plasminogen activator gene, pauA, in S. uberis was located between two ORFs with high homology to the DNA mismatch repair genes, hexA and hexB, and not on a DNA fragment between the genes encoding an ATP binding cassette transporter protein (abc) and a protein involved in the formation and degradation of guanosine polyphosphates (rel) as is the case for **streptokinase** in other streptococci.

L11 ANSWER 29 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1998:789174 HCAPLUS
DOCUMENT NUMBER: 130:24116
TITLE: Production of non-immunogenic proteins by removal of T-cell and B-cell epitopes
INVENTOR(S): Carr, Francis Joseph
PATENT ASSIGNEE(S): Biovation Ltd., UK
SOURCE: PCT Int. Appl., 77 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9852976	A1	19981126	WO 1998-GB1473	19980521
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2290485	AA	19981126	CA 1998-2290485	19980521
AU 9875393	A1	19981211	AU 1998-75393	19980521
AU 736549	B2	20010802		
GB 2339430	A1	20000126	GB 1999-25632	19980521
EP 983303	A1	20000308	EP 1998-922932	19980521
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002512624	T2	20020423	JP 1998-550129	19980521
US 2003153043	A1	20030814	US 2002-300215	20021120
PRIORITY APPLN. INFO.:			GB 1997-10480 A	19970521

GB 1997-16197	A	19970731
GB 1997-25270	A	19971128
US 1997-67235P	P	19971202
GB 1998-7751	A	19980414
WO 1998-GB1473	W	19980521
US 1999-438136	B1	19991110

AB Proteins, or parts of proteins, may be rendered non-immunogenic, or less immunogenic, to a given species by identifying in their amino acid sequences one or more potential epitopes for T-cells of that species. Once identified, these amino acid sequence are modified to eliminate one or more MHC class II-restricted T-cell epitopes. In similar fashion, B-cell epitopes may be removed if desirable. By this process the immunogenicity of the protein when exposed to the immune system of the given species is reduced or eliminated. Monoclonal antibodies and other Ig-like mols. can particularly benefit from being rendered less immunogenic (e.g., humanized antibodies for therapy).

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 30 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 10

ACCESSION NUMBER: 1998:317596 BIOSIS
DOCUMENT NUMBER: PREV199800317596
TITLE: Effect of signal peptide changes on the extracellular processing of **streptokinase** from Escherichia coli: Requirement for secondary structure at the cleavage junction.
AUTHOR(S): Pratap, J.; Dikshit, K. L. [Reprint author]
CORPORATE SOURCE: Inst. Microbial Technology, Sector 39-A, Chandigarh 160 036, India
SOURCE: Molecular and General Genetics, (May, 1998) Vol. 258, No. 4, pp. 326-333. print.
CODEN: MGGEAE. ISSN: 0026-8925.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 22 Jul 1998
Last Updated on STN: 10 Sep 1998

AB **Streptokinase** (SK), an extracellular protein from Streptococcus **equisimilis**, is secreted post-translationally by Escherichia coli using both its native and E. coli-derived transport signals. In this communication we report that cleavage specificity of signal peptidase I, and thus efficiency of secretion, varies in E. coli when SK export is directed by different transport signals. The native (+ 1) N-terminus of mature SK was retained when it was transported under the control of its own, PelB or LamB signal peptide. However, when translocation of SK was controlled by the OmpA or MalE signal peptide, Ala2 of mature SK was preferred as a cleavage site for the pre-SK processing. Our results indicate that compatibility of the leader peptide with the mature sequences of SK, which fulfils the requirement for a given secondary structure within the cleavage region, is essential for maintaining the correct processing of pre-SK. An OmpA-SK fusion, which results in the deletion of two N-terminal amino acid residues of mature SK, was further studied with respect to the recognition of alternative cleavage site in E. coli. The alanine at +2 in mature SK was changed to glycine or its relative position was changed to +3 by introducing a methionine residue at the +1 position. Both alterations resulted in the correct cleavage of pre-SK at the original OmpA fusion site. In contrast, introduction of an additional alanine at +4, creating three probable cleavage sites (Ala-x-Ala-x-Ala-x-Ala), resulted in the recognition of all three target sites for cleavage, with varying efficiency. The results indicate that the nature of the secondary structure generated at the cleavage junction of pre-SK, resulting from the fusion of different signal peptides, modulates the cleavage specificity of signal peptidase I during extracellular processing of SK. Based on these findings it is proposed

that flexibility in the interaction of the active site of signal peptidase I with the cleavage sites of signal peptides may occur when it encounters two or more juxtaposed cleavage sites. Preference for one cleavage site over another, then, may depend on fulfillment of secondary structure requirements in the vicinity of the pre-protein cleavage junction.

L11 ANSWER 31 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1999:317401 SCISEARCH
THE GENUINE ARTICLE: 189BZ
TITLE: Hydrophobic interaction chromatography applied to purification of **recombinant streptokinase**
AUTHOR: Perez N (Reprint); Urrutia E; Camino J; Orta D R; Torres Y; Martinez Y; Cruz M; Alburquerque S; Gil M R; Hernandez L
CORPORATE SOURCE: Ctr Genet Engn & Biotechnol, Streptokinase Div, Havana, Cuba; Ctr Genet Engn & Biotechnol, Qual Control Div, Havana, Cuba
COUNTRY OF AUTHOR: Cuba
SOURCE: MINERVA BIOTECNOLOGICA, (DEC 1998) Vol. 10, No. 4, pp. 174-177.
ISSN: 1120-4826.
PUBLISHER: EDIZIONI MINERVA MEDICA, CORSO BRAMANTE 83-85 INT JOURNALS DEPT., 10126 TURIN, ITALY.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 22
ENTRY DATE: Entered STN: 1999
Last Updated on STN: 1999
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background **Recombinant streptokinase** (rSk) is a streptococcal protein **cloned** in E. coli.(11) Several methods have been described for **streptokinase** purification: ion exchange chromatography,(12) affinity chromatography with canine plasmin(13) and chromatography on immobilized acylated human plasminogen.(14) Monoclonal antibodies anti-rSk immobilized to Sepharose(15) have been used too. Recently this protein was purified using HIC.

Methods. rSk (CIGB, Cuba) was produced by fermentation strain K12 of E. coli,(11) the protein was recovered after washed pellet, cellular disruption and solubilization. Several purification assays were done using TSK-Butyl (Tosohaas, Japan) as a support for hydrophobic interaction chromatography (HIC). The protein was loaded to the column with 1 M of ammonium sulfate before being washed using an elution gradient from 0.5 to 0 M of ammonium sulfate, in order to determine the elution point of the rSk.

Results. Wie could determine that this protein is partly hydrophobic, this determination was shown by analysis of its aminoacidic sequence. This protein has 415 aminoacids of which 36% are non polar. The absorption capacity for TSK Butyl 650 S varies from 15 to 20 mg/mL. The optimum elution point was obtained using 0.25 M of ammonium sulfate, the eluted material was obtained with a high level of purity (<1% of contaminants). The recovery of rSk was about 49% using the mean of five assays.

Conclusions. The experimental process evaluated could be efficiently inserted in a downstream process to obtain **recombinant streptokinase** highly purified as final preparation and good recovery.

L11 ANSWER 32 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1998:473042 SCISEARCH
THE GENUINE ARTICLE: ZV077
TITLE: The interaction of Streptococcus dysgalactiae with plasmin

and plasminogen
AUTHOR: Leigh J A (Reprint); Hodgkinson S M; Lincoln R A
CORPORATE SOURCE: Inst Anim Hlth, Compton Lab, Newbury RG20 7NN, Berks, England (Reprint)
COUNTRY OF AUTHOR: England
SOURCE: VETERINARY MICROBIOLOGY, (15 MAR 1998) Vol. 61, No. 1-2, pp. 121-135.
ISSN: 0378-1135.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 29
ENTRY DATE: Entered STN: 1998
Last Updated on STN: 1998
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The activation of plasminogen and the binding of plasmin by bacteria may have many effects which promote infection. The occurrence of such activities in streptococci is well documented; however, these are yet to be demonstrated for S. dysgalactiae. Consequently, the ability of this bacterium to activate mammalian plasminogen and bind either plasmin or its zymogen was investigated. Activation of bovine plasminogen was dependant on both the strain and the growth medium used for cultivation. Eighteen strains were able to activate bovine and ovine plasminogen and some of these also activated plasminogen from the horse, rabbit and pig. None activated human plasminogen and one strain (CE127) did not activate plasminogen from any source. Tricine-SDS PAGE and zymographic analysis of culture supernatants showed that bovine plasminogen was activated by four out of six strains at two locations corresponding to 16 kDa and IO kDa. Following the growth of five strains in the presence of bovine plasminogen, all but strain CE127 bound high levels of plasmin activity. In contrast, following growth in human plasminogen none of the strains exhibited bound plasmin activity although all could bind human plasmin directly. Ail strains were also able to bind bovine and human plasminogen in such a way as to allow its activation by urokinase. We conclude that S. dysgalactiae is capable of activating mammalian plasminogen in a species-specific fashion and that the bacterium is also capable of binding plasmin and plasminogen with an apparent preference for bovine plasmin over human plasmin and/or plasminogen from either species. (C) 1998 Elsevier Science B.V.

L11 ANSWER 33 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1998-11139 BIOTECHDS
TITLE: **Streptokinase**-mediated plasminogen activation: molecular studies using genetically engineered **streptokinase** variants; **recombinant streptokinase** production via plasmid pGEX-3X **expression** in Streptococcus pyogenes and Streptococcus **equisimilis**
AUTHOR: Lizano S
CORPORATE SOURCE: Univ.Costa-Rica
LOCATION: Instituto Clodomiro Picardo, Facultad de Microbiologia, and Departamento de Bioquimica, Escuela de Medicina, Universidad de Costa Rica, San Jose, Costa Rica.
SOURCE: Biotecnol.Apl.; (1998) 15, 2, 110
CODEN: 2048M
ISSN: 0864-4551
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Streptokinase** triggers a non-proteolytic activation of plasminogen by forming a stoichiometric complex with the plasminogen. Streptococcus pyogenes NZ131, SP13013 and Streptococcus **equisimilis** H46A strains were chosen for this study. After isolation of the **streptokinase** their sequences were amplified

using polymerase chain reaction. The amplification was followed by ligation into plasmid pUC18 and then subcloning into **expression** vector plasmid pGEX-3X in translational frame with the glutathione-transferase (EC-2.5.1.18) gene (GST). The cells were then transformed and colonies which **expressed** GST-**streptokinase** were selected. An internal polymorphic region of the enzyme molecule (implicated in pathogenesis) was deleted and replaced with a linker. Site-directed mutagenesis was performed and human Glu-plasminogen was purified and the **recombinant** constructs were assayed. This method provided an alternative method to preproteolysis plasminogen activation. Future efforts to characterize the structure/function relationship of **streptokinase** may influence its engineering and so improve its therapeutic potential and explain its role in disease. (7 ref)

L11 ANSWER 34 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:437021 SCISEARCH
THE GENUINE ARTICLE: ZU066
TITLE: The Streptococcus agalactiae hylB gene encoding hyaluronate lyase: completion of the sequence and **expression** analysis
AUTHOR: Gase K; Ozegowski J; Malke H (Reprint)
CORPORATE SOURCE: Univ Jena, Inst Mol Biol, Winzerlaer Str 10, D-07745 Jena, Germany (Reprint); Univ Jena, Inst Mol Biol, D-07745 Jena, Germany; Univ Jena, Inst Expt Microbiol, D-07745 Jena, Germany
COUNTRY OF AUTHOR: Germany
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA-GENE STRUCTURE AND EXPRESSION, (29 MAY 1998) Vol. 1398, No. 1, pp. 86-98. ISSN: 0167-4781.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 43
ENTRY DATE: Entered STN: 1998
Last Updated on STN: 1998

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We report the **cloning**, sequencing and **expression** analysis of the Streptococcus agalactiae strain 4755 hylB(4755) allele, the first chromosomally-encoded streptococcal hyaluronate lyase gene to be **cloned** and sequenced completely. This gene lies in a region homologous to that found in S. mutans, between the mutX and rmlB genes, a region involved in the synthesis of the serotype c-specific polysaccharide antigen of this organism. Sequencing of hylB(4755) revealed a 3216-bp open reading frame that encodes a 121.2-kDa polypeptide possessing a 30-amino acid signal sequence which was theoretically predicted and experimentally confirmed. A **recombinant** plasmid, pHYB100, containing hylB(4755) together with its promoter and terminator was constructed and used to analyze the **expression** of the gene in Escherichia coli. In Northern hybridization experiments, hylB(4755) was found to be transcribed as 3.3-kb monocistronic mRNA from its own promoter which exhibits an extended, sigma(70)-like 10 consensus sequence. Transcript mapping by primer extension analysis placed the major transcription initiation site leading to the longest transcript 38 bp upstream of the translational initiation codon: ATG. E. coli TG1(pHYB100) efficiently synthesized hyaluronan-cleaving enzyme activity at similar to 7000 working units/10(9) cells, with lyase activity detectable in all principle cellular locations. Zymography and Western analysis identified functional activity in TG1(pHYB100) to be associated with similar to 118, 110 and 94-kDa polypeptides, with the two low molecular weight species constituting the major components of the enzyme purified from the culture supernatant fluid of S. agalactiae 4755. The 118-kDa form was shown to

represent the undegraded mature enzyme, whereas the smaller species are likely to arise from proteolytic cleavage in the N-terminal part of the mature protein. The HylB(4755) protein showed extensive sequence identity to the homologous enzymes from S. agalactiae 3502 and S. pneumoniae characterized by others but sequence comparisons clearly show that incomplete genes truncated at their 5' ends had been isolated from these two organisms.

L11 ANSWER 35 OF 106 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 1998350778 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9686161
TITLE: **Cloning, expression** and purification of **recombinant streptokinase**: partial characterization of the protein **expressed** in Escherichia coli.
AUTHOR: Avilan L; Yarzabal A; Jurgensen C; Bastidas M; Cruz J; Puig J
CORPORATE SOURCE: Laboratorio de Biologia y Medicina Experimental, Facultad de Ciencias, Universidad de Los Andes, Merida, Venezuela.
SOURCE: Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica ... [et al.], (1997 Dec) 30 (12) 1427-30.
Journal code: 8112917. ISSN: 0100-879X.
PUB. COUNTRY: Brazil
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19981020
Last Updated on STN: 19981020
Entered Medline: 19981005

AB We **cloned** the **streptokinase** (STK) gene of Streptococcus **equisimilis** in an **expression** vector of Escherichia coli to overexpress the profibrinolytic protein under the control of a tac promoter. Almost all the **recombinant** STK was exported to the periplasmic space and recovered after gentle lysozyme digestion of induced cells. The periplasmic fraction was chromatographed on DEAE Sepharose followed by chromatography on phenyl-agarose. Active proteins eluted between 4.5 and 0% ammonium sulfate, when a linear gradient was applied. Three major STK derivatives of 47.5 kDa, 45 kDa and 32 kDa were detected by Western blot analysis with a polyclonal antibody. The 32-kDa protein formed a complex with human plasminogen but did not exhibit Glu-plasminogen activator activity, as revealed by a zymographic assay, whereas the 45-kDa protein showed a K(m) = 0.70 microM and kcat = 0.82 s-1, when assayed with a chromogen-coupled substrate. These results suggest that these proteins are putative fragments of STK, possibly derived from partial degradation during the export pathway or the purification steps. The 47.5-kDa band corresponded to the native STK, as revealed by peptide sequencing.

L11 ANSWER 36 OF 106 SCISEARCH
ACCESSION NUMBER: 1997:547160 SCISEARCH
THE GENUINE ARTICLE: XL484
TITLE: The LppC gene of Streptococcus **equisimilis** encodes a lipoprotein that is homologous to the e(P4) outer membrane protein from Haemophilus influenzae
AUTHOR: Gase K (Reprint); Liu G W; Bruckmann A; Steiner K; Ozegowski J; Malke H
CORPORATE SOURCE: UNIV JENA, INST MOL BIOL, D-07745 JENA, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: MEDICAL MICROBIOLOGY AND IMMUNOLOGY, (JUN 1997) Vol. 186, No. 1, pp. 63-73.

ISSN: 0300-8584.
PUBLISHER: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 39.
ENTRY DATE: Entered STN: 1997
Last Updated on STN: 1997
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We report the **cloning**, sequencing, and analysis of a novel chromosomal gene of Streptococcus **equisimilis** strain H46A that codes for a membrane lipoprotein, designated LppC. The lppC gene is located 3' adjacent to, and co-oriented with, the unrelated gapC gene that encodes the previously characterized glyceraldehyde-3-phosphate dehydrogenase. Sequencing of lppC revealed an 855-bp open reading frame that predicted a 32.4-kDa polypeptide possessing a potential lipoprotein signal sequence and modification site (VTGC). Signal sequence processing of LppC synthesized in the homologous host or **expressed** from plasmid pLPP2 in Escherichia coli was sensitive to globomycin, a selective inhibitor of lipoprotein-specific signal peptidase II. Subcellular localization of LppC using polyclonal antibodies raised to the hexahistidyl-tagged protein proved LppC to be tightly associated with the cytoplasmic membrane of S. **equisimilis** and with the outer membrane of E. coli JM109 (pLPP2). Southern, Northern and Western analyses indicated that Ipl, was conserved in S. pyogenes, and transcribed independently of gap as monocistronic 0.9-kb mRNA from a sigma(70)-like consensus promoter. Database searches found homology of LppC to the hel gene-encoded outer membrane protein e (P4) from Haemophilus influenzae to which it exhibits 58% sequence similarity. However, unlike the hel gene, lppC was unable to complement hemA mutants of E. coli for growth on hemin as sole porphyrin source in aerobic conditions. Furthermore, neither the wild type nor an lppC insertion mutant of S. **equisimilis** could grow on hemin in iron-limited medium. These results, together with findings indicating that S. **equisimilis** H46A had no absolute requirement for iron, led us to conclude that lppC, in contrast to hel, is not involved in hemin utilization and has yet to be assigned a function.

L11 ANSWER 37 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1997:155090 HCAPLUS
DOCUMENT NUMBER: 126:154444
TITLE: **Streptokinases** analogs resistant to cleavage and inactivation by plasmin
INVENTOR(S): Reed, Guy L.
PATENT ASSIGNEE(S): President and Fellows of Harvard College, USA
SOURCE: PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9641883	A1	19961227	WO 1996-US9640	19960607
W: CA, JP, MX				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5854049	A	19981229	US 1995-488940	19950609
PRIORITY APPLN. INFO.:			US 1995-488940 A	19950609

AB **Streptokinase** analogs with altered plasmin-binding features that are resistant to binding by plasmin and subsequent cleavage and inactivation are described for use as thrombolytics with a prolonged serum half-life. Specifically, analogs of the Streptococcus **equisimilis** **streptokinase** are described. Changes that increase plasminogen resistance include alterations of the plasmin-binding domain and and

blocking of the N-terminus. Fusion proteins with maltose-binding protein as the N-terminal moiety are prepared and their plasmin resistance and **streptokinase** activity are described. Similarly, analogs with substitutions of basic amino acids that identify internal plasmin and trypsin cleavage sites were prepared and characterized.

L11 ANSWER 38 OF 106 LIFESCI COPYRIGHT 2005 CSA on STN
ACCESSION NUMBER: 96:44932 LIFESCI
TITLE: Functional analysis of a relA/spoT gene homolog from Streptococcus **equisimilis**
AUTHOR: Mechold, U.; Cashel, M.; Steiner, K.; Gentry, D.; Malke, H.
CORPORATE SOURCE: Inst. Molecular Biol., Jena Univ., Winzerlaer Str. 10, D-07745 Jena, Germany
SOURCE: J. BACTERIOL., (1996) vol. 178, no. 5, pp. 1404-1411. ISSN: 0021-9193.
DOCUMENT TYPE: Journal
FILE SEGMENT: J; G
LANGUAGE: English
SUMMARY LANGUAGE: English
AB We examined the functional attributes of a gene encountered by sequencing the **streptokinase** gene region of Streptococcus **equisimilis** H46A. This gene, originally called rel, here termed rel sub( )S. **equisimilis**, is homologous to two related Escherichia coli genes, spoT and relA, that function in the metabolism of guanosine 5',3'-polyphosphates [(p)ppGpp]. Studies with a variety of E. coli mutants led us to deduce that the highly **expressed** rel sub( )S. **equisimilis** gene encodes a strong (p)ppGppase and a weaker (p)ppGpp synthetic activity, much like the spoT gene, with a net effect favoring degradation and no complementation of the absence of the relA gene. We verified that the Rel sub( )S. **equisimilis** protein, purified from an E. coli relA spoT double mutant, catalyzed a manganese-activated (p)ppGpp 3'-pyrophosphohydrolase reaction similar to that of the SpoT enzyme. This Rel sub( )S. **equisimilis** protein preparation also weakly catalyzed a ribosome-independent synthesis of (p)ppGpp by an ATP to GTP 3'-pyrophosphoryltransferase reaction when degradation was restricted by the absence of manganese ions. An analogous activity has been deduced for the SpoT protein from genetic evidence. In addition, the Rel sub( )S. **equisimilis** protein displays immunological cross-reactivity with polyclonal antibodies specific for SpoT but not for RelA. Despite assignment of rel sub( )S. **equisimilis** gene function in E. coli as being similar to that of the native spoT gene, disruptions of rel sub( )S. **equisimilis** in S. **equisimilis** abolish the parental (p)ppGpp accumulation response to amino acid starvation in a manner expected for relA mutants rather than spoT mutants.

L11 ANSWER 39 OF 106 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 96200111 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8631718
TITLE: Functional analysis of a relA/spoT gene homolog from Streptococcus **equisimilis**.
AUTHOR: Mechold U; Cashel M; Steiner K; Gentry D; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Journal of bacteriology, (1996 Mar) 178 (5) 1401-11. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960715
Last Updated on STN: 19970203
Entered Medline: 19960703
AB We examined the functional attributes of a gene encountered by sequencing

the **streptokinase** gene region of Streptococcus **equisimilis** H46A. This gene, originally called rel, here termed relS. **equisimilis**, is homologous to two related Escherichia coli genes, spoT and relA, that function in the metabolism of guanosine 5',3'-polyphosphates [(p)ppGpp]. Studies with a variety of E. coli mutants led us to deduce that the highly **expressed** rel S. **equisimilis** gene encodes a strong (p)ppGppase and a weaker (p)ppGpp synthetic activity, much like the spoT gene, with a net effect favoring degradation and no complementation of the absence of the relA gene. We verified that the Rel S. **equisimilis** protein, purified from an E. coli relA spoT double mutant, catalyzed a manganese-activated (p)ppGpp 3'-pyrophosphohydrolase reaction similar to that of the SpoT enzyme. This Rel S. **equisimilis** protein preparation also weakly catalyzed a ribosome-independent synthesis of (p)ppGpp by an ATP to GTP 3'-pyrophosphoryltransferase reaction when degradation was restricted by the absence of manganese ions. An analogous activity has been deduced for the SpoT protein from genetic evidence. In addition, the Rel S. **equisimilis** protein displays immunological cross-reactivity with polyclonal antibodies specific for SpoT but not for RelA. Despite assignment of rel S. **equisimilis** gene function in E. coli as being similar to that of the native spoT gene, disruptions of rel S. **equisimilis** in S. **equisimilis** abolish the parental (p)ppGpp accumulation response to amino acid starvation in a manner expected for relA mutants rather than spoT mutants.

L11 ANSWER 40 OF 106 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 96397500 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8804394
TITLE: **Cloning** of heterologous genes specifying detrimental proteins on pUC-derived plasmids in Escherichia coli.
AUTHOR: Muller J; van Dijl J M; Venema G; Bron S
CORPORATE SOURCE: Institut fur Molekularbiologie, Friedrich-Schiller-Universitat Jena, Germany.
SOURCE: Molecular & general genetics : MGG, (1996 Aug 27) 252 (1-2) 207-11.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 19961219
Entered Medline: 19961031

AB A system is described that enables the **cloning** of genes specifying detrimental proteins in Escherichia coli. The system is based on pUC plasmids and was developed for the **expression** of the Bacillus subtilis csaA gene, which is lethal when **expressed** at high levels. Suppressor strains that tolerate the presence of plasmids for high-level **expression** of csaA were isolated, which contained small cryptic deletion variants of the parental plasmid in high copy numbers. The cryptic plasmids consisted mainly of the pUC replication functions and lacked the csaA region and selectable markers. The co-resident, incompatible, cryptic plasmids enabled the maintenance of the csaA plasmids by reducing their copy number 20-fold, which resulted in a concomitant 3- to 7-fold reduction in the **expression** of plasmid-encoded genes. Strains carrying these cryptic endogenous plasmids proved to be useful for the construction of pUC-based **recombinant** plasmids carrying other genes, such as the **skc** gene of Streptococcus **equisimilis**, which cannot be **cloned** in high copy numbers in E. coli. Several strategies to reduce production levels of heterologous proteins specified by plasmids are compared.

L11 ANSWER 41 OF 106 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN DUPLICATE 14

ACCESSION NUMBER: 96259705 EMBASE
DOCUMENT NUMBER: 1996259705
TITLE: Localization of the sequence-determined DNA bending center upstream of the **streptokinase** gene **skc**.
AUTHOR: Gross S.; Gase K.; Malke H.
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Winzerlaer Strasse 10,D-07745 Jena, Germany
SOURCE: Archives of Microbiology, (1996) Vol. 166, No. 2, pp. 116-121.
ISSN: 0302-8933 CODEN: AMICCW
COUNTRY: Germany
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 960924
Last Updated on STN: 960924

AB DNA sequences upstream of the core promoter region of the **streptokinase** gene (**skc**) from Streptococcus **equisimilis** H46A increase **skc** transcription more than tenfold in vivo. This promoter upstream region contains a segment of intrinsically bent DNA, the precise location of which was determined experimentally by circular permutation analysis and theoretically by computer prediction. Electrophoretic analysis of circularly permuted upstream DNA fragments placed the bend center approximately at position -100 with respect to the major transcription initiation site of **skc**. This position was in excellent agreement with the center of maximum curvature predicted theoretically. Knowledge of the precise location of the bend center will be useful for future studies of the possible effect of DNA bending on **skc** transcription.

L11 ANSWER 42 OF 106 MEDLINE on STN DUPLICATE 15

ACCESSION NUMBER: 96305364 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8706717
TITLE: **Cloning**, sequencing and functional overexpression of the Streptococcus **equisimilis** H46A gapC gene encoding a glyceraldehyde-3-phosphate dehydrogenase that also functions as a plasmin(ogen)-binding protein. Purification and biochemical characterization of the protein.
AUTHOR: Gase K; Gase A; Schirmer H; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: European journal of biochemistry / FEBS, (1996 Jul 1) 239 (1) 42-51.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany; Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X97788
ENTRY MONTH: 199609
ENTRY DATE: Entered STN: 19960919
Last Updated on STN: 19990129
Entered Medline: 19960910

AB We previously identified DNA sequences involved in the function of the complex promoter of the **streptokinase** gene from Streptococcus **equisimilis** H46A, a human serogroup C strain known to **express** this gene at a high level. As a prerequisite to understanding possible mechanisms that control the balance between the plasminogen activating and plasmin(ogen) binding capacities of H46A, we describe here its gapC gene encoding glyceraldehyde-3-phosphate

dehydrogenase (GraP-DH, EC 1.2.1.12), a glycolytic enzyme apparently transported to the cell surface where it functions as a plasmin(ogen).binding protein. The gapC gene was **cloned** and sequenced and found to code for a 336-amino-acid polypeptide (approximately 35.9 kDa) exhibiting 94.9% sequence identity to the Plr protein from Streptococcus pyogenes shown by others to be capable of plasmin binding [Lottenberg, R., Broder, C. C., Boyle, M. D., Kain, S. J., Schroeder, B. L. & Curtiss, R. III (1992) J. Bacteriol. 174, 5204-5210]. To study the properties of the GapC protein, its gene was inducibly overexpressed in Escherichia coli from QIAexpress **expression** plasmids to yield the authentic GapC or (His)6GapC carrying a hexahistidyl N-terminus to permit affinity purification. Both proteins were functionally active, exhibiting specific GraP-DH activities of about 80 kat/mol (approximately 130 U/mg) after purification. Their binding parameters [association (ka) and dissociation (kd) rate constants, and equlibrium dissociation constants (Kd = kd/ka)] for the interaction with human Gluplasminogen and plasmin were determined by real-time biospecific interaction analysis using the Pharmacia BIAcore instrument. For comparative purposes, the commercial GraP-DH from Bacillus stearothermophilus (BstGraP-DH), a nonpathogenic organism, was included in these experiments. The Kd values for binding of plasminogen to GapC, (His)6GapC and BstGraP-DH were 220 nM, 260 nM and 520 nM, respectively, as compared to 25 nM, 17 nM and 98 nM, respectively, for the binding to plasmin. These data show that both the zymogen and active enzyme possess low-affinity binding sites for the gapC gene product and that the hexahistidyl terminus does not affect its function. Prior limited treatment with plasmin enhanced the subsequent plasminogen binding capacity of all three GraP-DHs, presumably by the exposure of new C-terminal lysine residues for binding to the zymogen.

L11 ANSWER 43 OF 106 MEDLINE on STN DUPLICATE 16
ACCESSION NUMBER: 96396845 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8803948
TITLE: Structural dissection and functional analysis of the complex promoter of the **streptokinase** gene from Streptococcus **equisimilis** H46A.
AUTHOR: Grafe S; Ellinger T; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Medical microbiology and immunology, (1996 May) 185 (1) 11-7.
Journal code: 0314524. ISSN: 0300-8584.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970131

AB The overlapping tandem promoters of the **streptokinase** gene, P1 and P2, identified previously by S1 nuclease transcript mapping were functionally dissected by mutagenesis of their -10 regions and fused transcriptionally with or without the 202-bp upstream region (USR) to the luciferase reporter gene (luc) from Photinus pyralis to analyze the contribution of the different sequence elements to promoter activity in Escherichia coli and the homologous Streptococcus **equisimilis** strain H46A. In E. coli, virtually the entire promoter activity derived from the upstream promoter P1. In S. **equisimilis**, luc **expression** increased in the following order of the involved sequence elements: P2 approximately equal to P2 + USR < P1 < P1 + P2 < P1 + USR < P1 + P2 + USR. This shows that (1) in the homologous system, P1 and P2 alone are extremely weak, (2) in the USR-less arrangement, only the combined core promoters have substantial activity, and (3) the USR stimulates only P1 and the combination of P1 + P2. Thus, the tandem

promoters presumably function by mutual contributary action and their full activity strongly depends on the AT-rich and statically bent upstream region. The distinctive feature determining the strength of P1 in both hosts appears to be its extended -10 region which matches the consensus TRTGN established for strong S. pneumoniae and Bacillus subtilis promoters.

L11 ANSWER 44 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:433656 HCAPLUS
DOCUMENT NUMBER: 133:27355
TITLE: **Cloning** and **expression** of Streptococcus H46 **streptokinase** gene
INVENTOR(S): Cho, Jung-Myong; Park, Yong-U.
PATENT ASSIGNEE(S): LG Chemical Co., Ltd., S. Korea
SOURCE: Repub. Korea, No pp. given
CODEN: KRXXFC
DOCUMENT TYPE: Patent
LANGUAGE: Korean
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
KR 9512901	B1	19951023	KR 1992-17406	19920924

PRIORITY APPLN. INFO.: KR 1992-17406 19920924
AB The **cloning** of **streptokinase** gene of Streptococcus H46 consists of PCR with primers and **cloning** the gene into the PstI-NdeI site of plasmid ptrp322H-HGH (KFCC 10067) to get ptrpH-SK (ATCC 68884). The DNA sequence of Streptococcus H46 **streptokinase** has 92.2-98.8% homol. to **SKC**, SKG, and SKA. Streptococcus H46 is also designated S. **equisimilis** ATCC 35556.

L11 ANSWER 45 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1996:90928 BIOSIS
DOCUMENT NUMBER: PREV199698663063
TITLE: Genetic modification of the **streptokinase** gene to study the interaction of **streptokinase** with plasminogen.
AUTHOR(S): Lizano, S.; Johnston, K. H. [Reprint author]
CORPORATE SOURCE: Dep. Microbiol. Immunol. Parasitol., La. State Univ. Med. Cent., 1901 Perdido St., New Orleans, LA 70112, USA
SOURCE: Ferretti, J. J. [Editor]; Klaenhammer, T. R. [Editor]; Brown, F. [Editor]; Gilmore, M. S. [Editor]. Dev. Biol. Stand., (1995) pp. 195-198. Developments in Biological Standardization; Genetics of streptococci, enterococci and lactococci.
Publisher: S. Karger AG, P.O. Box, Allschwilerstrasse 10, CH-4009 Basel, Switzerland; S. Karger AG, New York, New York, USA. Series: Developments in Biological Standardization.
Meeting Info.: 4th International American Society for Microbiology Conference on Streptococcal Genetics. Santa Fe, New Mexico, USA. May 15-18, 1994.
CODEN: DVBSA3. ISSN: 0301-5149. ISBN: 3-8055-6207-1.
DOCUMENT TYPE: Book
Conference; (Meeting)
Book; (Book Chapter)
Conference; (Meeting Paper)
LANGUAGE: English
ENTRY DATE: Entered STN: 4 Mar 1996
Last Updated on STN: 4 Mar 1996

L11 ANSWER 46 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on

STN
ACCESSION NUMBER: 1996:90927 BIOSIS
DOCUMENT NUMBER: PREV199698663062
TITLE: The **streptokinase** gene: Allelic variation, genomic environment and **expression** control.
AUTHOR(S): Malke, H. [Reprint author]; Steiner, K.; Gase, K.; Mechold, U.; Ellinger, T.
CORPORATE SOURCE: Inst. Mol. Biol., Jena Univ., Winzerlaer Str. 10, D-07745 Jena, Germany
SOURCE: Ferretti, J. J. [Editor]; Klaenhammer, T. R. [Editor]; Brown, F. [Editor]; Gilmore, M. S. [Editor]. Dev. Biol. Stand., (1995) pp. 183-193. Developments in Biological Standardization; Genetics of streptococci, enterococci and lactococci.
Publisher: S. Karger AG, P.O. Box, Allschwilerstrasse 10, CH-4009 Basel, Switzerland; S. Karger AG, New York, New York, USA. Series: Developments in Biological Standardization.
Meeting Info.: 4th International American Society for Microbiology Conference on Streptococcal Genetics. Santa Fe, New Mexico, USA. May 15-18, 1994.
CODEN: DVBSA3. ISSN: 0301-5149. ISBN: 3-8055-6207-1.
DOCUMENT TYPE: Book
Conference; (Meeting)
Book; (Book Chapter)
Conference; (Meeting Paper)
LANGUAGE: English
ENTRY DATE: Entered STN: 4 Mar 1996
Last Updated on STN: 4 Mar 1996

L11 ANSWER 47 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1995:547506 BIOSIS
DOCUMENT NUMBER: PREV199698561806
TITLE: High-level **expression** and secretion of **streptokinase** in Escherichia coli.
AUTHOR(S): Ko, Jae Hyeong; Park, Do Deun; Kim, Il Chul; Lee, Si Hyoung; Byun, Si Myung [Reprint author]
CORPORATE SOURCE: Dep. BioSci., Korea Advanced Inst. Sci. Technol., 373-1, Kusung-dong, Yusung-ku, Taejeon 305-701, South Korea
SOURCE: Biotechnology Letters, (1995) Vol. 17, No. 10, pp. 1019-1024.
CODEN: BILED3. ISSN: 0141-5492.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 31 Dec 1995
Last Updated on STN: 31 Dec 1995
AB The high-level **expression** plasmid for **streptokinase**, pSK100, has been constructed. It contains a tac promoter, an ompA signal sequence, a **streptokinase** structural gene(**skc**) and a rrnBT1T2 transcription terminator. E. coli JM 109 carrying pSK100 produced about 5,000IU of **streptokinase** per 1 ml of LB-ampicillin media. About 95% of the **expressed** **streptokinase** was secreted into the periplasmic and extracellular fractions. The **recombinant** **streptokinase** in high yield and purity may be a potential alternative source for the therapeutic agent.

L11 ANSWER 48 OF 106 MEDLINE on STN DUPLICATE 17
ACCESSION NUMBER: 95342169 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7616967
TITLE: Complex transcriptional control of the **streptokinase** gene of Streptococcus **equisimilis** H46A.

AUTHOR: Gase K; Ellinger T; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Molecular & general genetics : MGG, (1995 Jun 25) 247 (6) 749-58.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950905
Last Updated on STN: 19950905
Entered Medline: 19950822

AB On the Streptococcus **equisimilis** H46A chromosome, the divergent coding sequences of the genes for the plasminogen activator **streptokinase** (**skc**) and a leucine-rich protein (lrp), the function of which is unknown, are separated by a 328 bp intrinsically bent DNA region rich in AT tracts. To begin to understand the **expression** control of these two genes, we mapped their transcriptional initiation sites by S1 nuclease analysis and studied the influence of the bent intergenic region on promoter strength, using promoter-reporter gene fusions of **skc**' and lrp' to 'lacZ from Escherichia coli. The major transcriptional start sites, in both S. **equisimilis** and E. coli, mapped 22 bases upstream of the ATG start site of lrp (G), and 24 and 32 bases upstream of the translational initiation codon of **skc** (A and G, respectively), indicating the existence of two overlapping canonical **skc** promoters arranged in tandem on opposite faces of the helix. The reporter gene fusions were **cloned** in E. coli on a vector containing a 1.1 kb fragment of the S. **equisimilis** dexB gene, thus allowing promoter strength to be measured in multiple plasmid-form copies in the heterologous host and in single-copy genomic form following integration into the **skc** region of the homologous host. In S. **equisimilis**, **skc** '-'lacZ was **expressed** about 200-fold more strongly than the corresponding lrp'-'lacZ fusion. In contrast, in E. coli, the corresponding levels of **expression** differed by only about 11-fold. Deletion of the 202 bp bent region upstream of the **skc** and lrp core promoters caused a 13-fold decrease in **skc** promoter activity in S. **equisimilis** but did not alter lrp promoter strength in this host. In contrast, when studied in E. coli, this deletion did not alter the strength of the **skc**-double promoter and even increased by 2.4- to 3-fold the activity of the lrp promoter. This comparative promoter analysis shows that **skc** has a complex promoter structure, the activity of which in the homologous genomic environment specifically depends on sequences upstream of the two core promoters. Thus, the **skc** promoter structure resembles that of an array of promoters involved in a transcriptional switch; however, the nature of the potential switch factor(s) remains unknown.

L11 ANSWER 49 OF 106 MEDLINE on STN DUPLICATE 18
ACCESSION NUMBER: 95157528 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7531815
TITLE: Transcription termination of the **streptokinase** gene of Streptococcus **equisimilis** H46A: bidirectionality and efficiency in homologous and heterologous hosts.
AUTHOR: Steiner K; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Molecular & general genetics : MGG, (1995 Feb 6) 246 (3) 374-80.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 199503
ENTRY DATE: Entered STN: 19950322
Last Updated on STN: 19960129
Entered Medline: 19950316

AB In Streptococcus **equisimilis** H46A, a hypersymmetrical transcription terminator with bidirectional activity was localized between the translational termination codons of the **streptokinase** gene, **skc**, and the rel-orf1 genes. These two transcription units are oriented towards each other, and under normal conditions the **skc** mRNA level exceeds that of the rel-orf1 genes by a factor of at least 1000. Reporter vectors based on the promoterless cat gene were constructed by transcriptional fusion of **skc** to cat, such that the region between the two genes contained the terminator in **skc** orientation or in rel-orf1 orientation. Additionally, **skc** and cat were fused directly, with deletion of the terminator. The reporter vectors were designed to be capable of being studied either as multicopy plasmids in Escherichia coli or in single copy following integration, via **skc**, into the S. **equisimilis** chromosome.
Chloramphenicol acetyl transferase (CAT) activity assays in conjunction with determination of chloramphenicol resistance levels and Northern hybridization analysis showed that the terminator is active in either host and orientation. However, termination efficiency was host dependent, with high terminator strength being observed in the homologous streptococcal background and appreciable readthrough occurring in E. coli. The extent of transcriptional readthrough was dependent upon terminator orientation, with termination being more efficient in rel-orf1 polarity. The results suggest that, in S. **equisimilis**, transcription of both **skc** and rel-orf1 is efficiently terminated by a common signal, and that these genes are largely protected from convergent transcription, which otherwise would seem to be particularly detrimental to the weakly **expressed** rel-orf1 genes.

L11 ANSWER 50 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1995:622543 SCISEARCH
THE GENUINE ARTICLE: RU780
TITLE: **STREPTOKINASE**-MEDIATED PLASMINOGEN ACTIVATION USING A **RECOMBINANT** DUAL FUSION PROTEIN CONSTRUCT - A NOVEL-APPROACH TO STUDY BACTERIAL HOST PROTEIN INTERACTIONS
AUTHOR: LIZANO S (Reprint); JOHNSTON K H
CORPORATE SOURCE: LOUISIANA STATE UNIV, MED CTR, DEPT MICROBIOL IMMUNOL & PARASITOL, NEW ORLEANS, LA 70112
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF MICROBIOLOGICAL METHODS, (SEP 1995) Vol. 23, No. 3, pp. 261-280.
ISSN: 0167-7012.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 59
ENTRY DATE: Entered STN: 1995
Last Updated on STN: 1995
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Streptokinase** (SK), a plasminogen (Pg) activator secreted by groups A, C, and G streptococci, is extensively used as a pharmacological agent in thrombolytic therapy and possibly plays a role in streptococcal invasiveness and disease. SK activates Pg to plasmin (Ps) by forming an activator complex with Pg. However, the molecular basis whereby SK binds and activates Pg remains unclear, in part due to the rapid fragmentation of the SK-Pg complex. This study describes a solid phase approach to

study this interaction in which a **recombinant** SK molecule was constructed with glutathione-S-transferase appended to the NH2 terminus and (Gly)(3)(His)(8) appended to the COOH terminus. This dual fusion protein molecule, immobilized on either Sepharose-S-hexylglutathione or Ni2+ dinitriloacetic acid-Sepharose was then used to study the interaction of SK with Pg. These SK-Pg complexes exhibited amidolytic and proteolytic activity similar to native SK, but the pattern of fragmentation of the SK molecule was dependent upon whether the SK molecule was immobilized either at its NH2- or COOH terminus. This solid phase approach may contribute to a greater understanding of the role of SK in Pg activation by enabling the 'capture' of intact activator complexes under physiological conditions and, in addition, may serve as a useful model to analyze other bacterial-host protein interactions important in the pathogenesis of disease.

L11 ANSWER 51 OF 106 MEDLINE on STN DUPLICATE 19
ACCESSION NUMBER: 96157065 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8586173
TITLE: The **streptokinase** gene: allelic variation, genomic environment and **expression** control.
AUTHOR: Malke H; Steiner K; Gase K; Mechold U; Ellinger T
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Developments in biological standardization, (1995) 85 183-93. Ref: 36
Journal code: 0427140. ISSN: 0301-5149.
PUB. COUNTRY: Switzerland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199603
ENTRY DATE: Entered STN: 19960404
Last Updated on STN: 19960404
Entered Medline: 19960328

AB The genes for **streptokinase**, the most important prokaryotic plasmingoen activator, exhibit allelic variation predominantly due to the polymorphism of an internal 220-base pair fragment that divides the phylogenetic tree of their products into two primary branches. Current molecular genetic research seeks functional correlates of the allelic variation, aims at analyzing the genomic environment of the **streptokinase** gene, **skc**, and focuses on understanding its **expression**. Of the six genes **cloned** and sequenced in the **skc** region of Streptococcus **equisimilis** H46A, **skc** is **expressed** most abundantly in a fashion that involves two overlapping core promoters and upstream sequences rich of AT tracts. Transcription of **skc** is terminated at a hypersymmetrical site that functions bidirectionally and prevents convergent transcription of the oppositely oriented **skc** and rel-orf1 genes whose mRNA abundance differs by a factor of at least three orders of magnitude.

L11 ANSWER 52 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1995:290311 BIOSIS
DOCUMENT NUMBER: PREV199598304611
TITLE: **Cloning, Expression** and Sequence Analysis of **Streptokinases** from Streptococcus **equisimilis** which Preferentially Activate Equine and Porcine Plasminogens.
AUTHOR(S): Caballero, A. [Reprint author]; Johnston, K. H. [Reprint author]; Lottenberg, R.
CORPORATE SOURCE: LSU Med. Ctr., New Orleans, LA, USA
SOURCE: Abstracts of the General Meeting of the American Society

for Microbiology, (1995) Vol. 95, No. 0, pp. 172.
Meeting Info.: 95th General Meeting of the American Society for Microbiology. Washington, D.C., USA. May 21-25, 1995.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 5 Jul 1995
Last Updated on STN: 5 Jul 1995

L11 ANSWER 53 OF 106 MEDLINE on STN DUPLICATE 20
ACCESSION NUMBER: 96154934 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8577315
TITLE: Conservation of the organization of the **streptokinase** gene region among pathogenic streptococci.
AUTHOR: Frank C; Steiner K; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Medical microbiology and immunology, (1995 Oct) 184 (3) 139-46.
Journal code: 0314524. ISSN: 0300-8584.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X72832
ENTRY MONTH: 199603
ENTRY DATE: Entered STN: 19960321
Last Updated on STN: 19960321
Entered Medline: 19960313

AB Using ten gene-specific probes from the **cloned** and sequenced **streptokinase** gene (**skc**) region (8,931 bp) of Streptococcus **equisimilis** H46A, a human serogroup C strain, the conservation of these genes and their linkage relationships were studied by Southern hybridization in pathogenic streptococci differing taxonomically, serologically, in regard to their host range, and in the class of plasminogen activator produced. The results indicate that in S. pyogenes (strains A374, NZ131 and SF130/13) and a human group G strain (G19,908) both gene content and gene order as determined for H46A (dexB-abc-lrp-**skc**-orf1-rel) are preserved. The same is true of an equine S. **equisimilis** isolate (87-542-W), the **streptokinase** gene of which has been shown to hybridize detectably with **skc**, a result at variance with that obtained previously by others. In contrast, the chromosomal DNA of three S. uberis strains (0140J, C198, C216) of bovine origin, two of which produced a plasminogen activator different from **streptokinase**, hybridized only with dexB-, abc- and rel-specific probes, and the homologues of these genes appeared to lie close to each other. The maintenance of the organization of the **streptokinase** gene region in strains differing in overall chromosomal character suggests that this gene arrangement is of selective advantage.

L11 ANSWER 54 OF 106 MEDLINE on STN DUPLICATE 21
ACCESSION NUMBER: 96001243 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7557478
TITLE: Secretion of **streptokinase** fusion proteins from Escherichia coli cells through the hemolysin transporter.
AUTHOR: Kern I; Ceglowski P
CORPORATE SOURCE: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa.
SOURCE: Gene, (1995 Sep 22) 163 (1) 53-7.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199511
ENTRY DATE: Entered STN: 19951227
Last Updated on STN: 20021008
Entered Medline: 19951108

AB The hemolysin (HlyA) secretion system was used to achieve the sec-independent secretion of **streptokinase** (**Skc**) originating from Streptococcus **equisimilis** into the medium by Escherichia coli cells. The in-frame fusions of the **skc** gene, either possessing or lacking a region encoding the signal peptide (SP) with the 3'-end of the hlyA gene of various lengths were analysed. All hybrids retained **Skc** activity. Hybrid proteins devoided of the N-terminal SP, regardless of length of the hlyA secretion signal (62 vs. 194 amino acids), were secreted into the medium by the E. coli HlyA transporter at similar levels. Considerable amounts of hybrid proteins were still, however, associated with E. coli cells, mainly in the degraded form.

L11 ANSWER 55 OF 106 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1996:43901 SCISEARCH
THE GENUINE ARTICLE: TN446
TITLE: **Cloning** and sequencing of the **streptokinase** gene from Streptococcus pyogenes (CIP 56.57)
AUTHOR: Ball M M (Reprint); Puig J; Iborra F
CORPORATE SOURCE: UNIV PARIS 11, F-91405 ORSAY, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: DNA SEQUENCE, (1995) Vol. 6, No. 1, pp. 33-36.
ISSN: 1042-5179.
PUBLISHER: HARWOOD ACAD PUBL GMBH, C/O STBS LTD, PO BOX 90, READING, BERKS, ENGLAND RG1 8JL.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 10
ENTRY DATE: Entered STN: 1996
Last Updated on STN: 1996
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The **streptokinase** gene of the Streptococcus pyogenes strain CIP 56.57 was **cloned** and sequenced. This sequence coding for a 441 amino acid protein is well conserved among streptococcus species: there are two very conserved domains separated by a more variable region.

L11 ANSWER 56 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 22
ACCESSION NUMBER: 1994-07329 BIOTECHDS
TITLE: DNA encoding a plasminogen binding protein; **recombinant streptokinase** fragment production using new vector plasmid pMAL and a monoclonal antibody for use in myocardial infarction therapy
PATENT ASSIGNEE: Gen.Hosp.Boston; Univ.Harvard
PATENT INFO: WO 9407992 14 Apr 1994
APPLICATION INFO: WO 1993-US9502 5 Oct 1993
PRIORITY INFO: US 1993-128299 29 Sep 1993; US 1992-956692 5 Oct 1992
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1994-135561 [16]

AB DNA (I) encoding a **streptokinase** fragment (II) of residues 14-414 of a disclosed protein sequence is claimed. (II) does not contain residues 244-352, but may contain residues 1-352, 120-352, 244-414 or 244-352 of the protein sequence. Also claimed are: (1) an **expression** vector containing (I); (2) a host cell transformed

with the vector of (1); (3) (II) encoded by (I); (4) detecting plasminogen in a biological sample by contacting the sample with (II) and detecting any (II)-plasminogen complex formed; (5) a method for assaying (II) for antigenicity involving contacting (II) with a monoclonal antibody specific for a distinct epitope of **streptokinase** and determining whether the fragments bind to the MAb and, optionally, also whether the fragments can activate plasminogen in the presence of the MAb; (6) a method for myocardial infarction therapy involving administering (II) to a patient; and (7) a monoclonal antibody specific for a distinct epitope of **streptokinase**. In an example, (II) genes from Streptococcus **equisimilis** were fused with maltose binding protein genes and **expressed** in Escherichia coli using plasmid pMAL. (62pp)

L11 ANSWER 57 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 23
ACCESSION NUMBER: 1994-04368 BIOTECHDS
TITLE: Engineering and production of **streptokinase** in a Bacillus subtilis **expression**-secretion system; Streptococcus **equisimilis** gene **cloning**, protein secretion and protein engineering for improved C-terminus stability in a protease-deficient strain
AUTHOR: Wong S L; Ye R; Nathoo S
CORPORATE SOURCE: Univ.Calgary
LOCATION: Department of Biological Sciences, The University of Calgary, 2500 University Drive, N.W., Calgary, Alberta T2N 1N4, Canada.
SOURCE: Appl.Environ.Microbiol.; (1994) 60, 2, 517-23
CODEN: AEMIDF
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Production of Streptococcus **equisimilis** H46A secreted **recombinant streptokinase** from various Bacillus subtilis strains was studied. The **skc** gene was **cloned** and **expressed** using plasmid pSK-1 (with the intact **skc** gene and its promoter region) or plasmid pSK-3 (with the mature portion of **streptokinase** fused to the B. subtilis levansucrase sacB gene promoter and signal peptide sequence) as vector. The use of the 6-extracellular-protease-deficient strain B. subtilis WB600 as host greatly improved the production yield. B. subtilis carrying either the wild-type or modified **skc** gene produced **streptokinase** at a comparable level. Even with WB600 as host, a C-terminally-processed **streptokinase** was also produced. Through region-specific combinatorial mutagenesis around the C-terminal processing sites, **streptokinase** derivatives resistant to C-terminal degradation were engineered. Plasmid pSKC-27 encoded a derivative which showed a 2.5-fold increase in specific activity, which should make it a more favorable thrombolytic agent. (35 ref)

L11 ANSWER 58 OF 106 MEDLINE on STN DUPLICATE 24
ACCESSION NUMBER: 94178706 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8132150
TITLE: Inactivation of the **streptokinase** gene prevents Streptococcus **equisimilis** H46A from acquiring cell-associated plasmin activity in the presence of plasminogen.
AUTHOR: Malke H; Mechold U; Gase K; Gerlach D
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, FRG.
SOURCE: FEMS microbiology letters, (1994 Feb 1) 116 (1) 107-12.
Journal code: 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 199404
ENTRY DATE: Entered STN: 19940428
Last Updated on STN: 19940428
Entered Medline: 19940418

AB The **streptokinase** gene of Streptococcus **equisimilis** H46 was inactivated by plasmid insertion mutagenesis to study the relationship between elaboration of **streptokinase** and acquisition of cell-associated plasmin activity after incubation of wild-type and mutant cells in media containing plasminogen or plasmin. The results showed that H46A binds both the zymogen and active enzyme, generates surface-associated plasmin activity in the presence of plasminogen when producing **streptokinase**, and **expresses** its plasmin(ogen) receptor(s) independently of a functional **streptokinase** gene. At least part of the plasmin(ogen) binding capacity may be due to the glyceraldehyde-3-phosphate dehydrogenase type of receptor molecule, as judged by the detection of the corresponding gene.

L11 ANSWER 59 OF 106 MEDLINE on STN DUPLICATE 25
ACCESSION NUMBER: 94049672 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8232196
TITLE: Genetic organization of the **streptokinase** region of the Streptococcus **equisimilis** H46A chromosome.
AUTHOR: Mechold U; Steiner K; Vettermann S; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Molecular & general genetics : MGG, (1993 Oct) 241 (1-2) 129-40.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X72832
ENTRY MONTH: 199312
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 19940117
Entered Medline: 19931215

AB The complete nucleotide sequences of four genes and one open reading frame (ORF1) adjacent to the **streptokinase** gene, **skc**, from Streptococcus **equisimilis** H46A were determined. These genes are encoded on the opposite DNA strand to **skc** and are arranged as follows: dexB-abc-lrp-**skc**-ORF1-rel. The dexB gene, coding for an alpha-glucosidase (M(r) 61,733), and abc, encoding an ABC transporter (M(r) 42,080), are similar to the dexB and msmK genes, respectively, from the multiple sugar metabolism operon of S. mutans. The lrp gene specifies a leucine-rich protein (M(r) 32,302) that has a leucine-zipper motif at its C-terminus. The function of the Lrp protein is not known but appeared to be detrimental when overexpressed in Escherichia coli. Although lrp appears not to be an essential gene, as judged by plasmid insertion mutagenesis, it is conserved in all streptococcal strains carrying a **streptokinase** gene. The rel gene showed significant homology to the E. coli relA and spoT genes involved in the stringent response to amino acid deprivation. Multiple alignment of the amino acid sequences of Rel (M(r) 83,913), RelA and SpoT revealed 59.4% homology of the primary structures. Northern hybridization analyses of the genes in the **skc** region showed **skc** to be transcribed most abundantly. In addition to transcripts for **skc**, monocistronic mRNAs were detected for all three genes divergently transcribed from **skc**. Although there was also some read-through transcription from lrp into abc, and from abc into dexB, the transcription pattern suggests a high degree of transcriptional and functional independence not only of **skc** but also abc and dexB. Prominent structural features in intergenic regions included a static DNA bending locus located upstream and a putative bidirectional transcription terminator downstream of **skc**

L11 ANSWER 60 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1992:441935 HCAPLUS
DOCUMENT NUMBER: 117:41935
TITLE: **Cloning** and **expression** of **streptokinase** gene of C-type Streptococcus **equisimilis**
PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba
SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 04030794	A2	19920203	JP 1990-201600	19900731
JP 3127298	B2	20010122		
EP 489201	A1	19920610	EP 1990-201930	19900717
EP 489201	B1	19951115		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 130369	E	19951215	AT 1990-201930	19900717
ES 2081909	T3	19960316	ES 1990-201930	19900717
US 5296366	A	19940322	US 1991-703778	19910522
AU 644657	B2	19931216	AU 1991-78101	19910531
RU 2107726	C1	19980327	RU 1991-5001280	19910717
PRIORITY APPLN. INFO.:			CU 1990-90 A	19900523
			SU 1991-5001280 A	19910717

AB The **streptokinase** (I) gene **SKC-2** ,with/without signal sequence, is **cloned** from C-type S. **equisimilis** ATCC-9542 by the polymerase chain reaction method and **expressed** in Escherichia coli and yeast for com. manufacture of I. Genomic DNA of the C-type S. **equisimilis** was isolated by the standard method and amplified with primers derived from the nucleotide sequence of **SKC** to get I gene with/without signal sequence. **Expression** of the I gene in E. coli and Pichia pastoris MP-36 mutant were shown. The production of I with these microorganisms were ≥350 mg/L and ≥1.2 g/L, resp.

L11 ANSWER 61 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1993:229181 HCAPLUS
DOCUMENT NUMBER: 118:229181
TITLE: **Cloning** and **expression** of a gene for **streptokinase** from a hemolytic Streptococcus
INVENTOR(S): Garcia, Mario P. E.; Chaplen, Roger R.; Hidalgo, Aimee P.; Doce, Ricardo S.; Marrero, Luciano F. H.; Collazo, Pedro R.; Ramirez, Anaisel C.; Munoz, Emilio A. M.; Martinez, Walfrido B.; et al.
PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba
SOURCE: Can. Pat. Appl., 26 pp.
CODEN: CPXXEB
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CA 2043953	AA	19921206	CA 1991-2043953	19910605

CA 2043953	C	20010612		
HU 62655	A2	19930528	HU 1991-1770	19910527
HU 216073	B	19990428		
SK 279873	B6	19990507	SK 1991-2256	19910719

PRIORITY APPLN. INFO.: CA 1991-2043953 19910605

AB The gene for **streptokinase** of a Streptococcus **equisimilis** type C is **cloned** and **expressed** in Escherichia coli or in yeasts. **Expression** in yeasts uses the promoter of the AOX1 gene of Pichia pastoris to regulate **expression**. Secretion of the protein was achieved using the cognate signal peptide or one from sucrose invertase. The gene was **cloned** by PCR amplification using different pairs of primers to **clone** the gene with or without the signal sequence. Integrating **expression** vectors for **expression** of the gene in Pichia pastoris with or without secretion of the product were constructed. When the secretory construct was used, **streptokinase** yields of 1-1.2 g/L were obtained. The protein had the expected biol. activities, and the purified enzyme had a specific activity of 50,000-100,000 units/mg.

L11 ANSWER 62 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1992:340953 BIOSIS
DOCUMENT NUMBER: PREV199243030503; BR43:30503
TITLE: **STREPTOKINASE** MUTATIONS AFFECTING **SKC EXPRESSION** IN HOMOLOGOUS AND HETEROLOGOUS HOSTS.
AUTHOR(S): MECHOLD U [Reprint author]; MULLER J; MALKE H
CORPORATE SOURCE: CENTRAL INST MICROBIOL EXP THERAPY, JENA D-6900, GER
SOURCE: Zentralblatt fuer Bakteriologie Supplement, (1992) pp. 336-338. OREFICI, G. (ED.). ZENTRALBLATT FUER BAKTERIOLOGIE SUPPLEMENT, 22. NEW PERSPECTIVES ON STREPTOCOCCI AND STREPTOCOCCAL INFECTIONS; (INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY, 22. NEW PERSPECTIVES ON STREPTOCOCCI AND STREPTOCOCCAL INFECTIONS); XI LANCEFIELD INTERNATIONAL SYMPOSIUM ON STREPTOCOCCI AND STREPTOCOCCAL DISEASES, SIENA, ITALY, SEPTEMBER 10-14, 1990. XIX+569P. GUSTAV FISCHER VERLAG: STUTTGART, GERMANY; NEW YORK, NEW YORK, USA. ILLUS.
Publisher: Series: Zentralblatt fuer Bakteriologie Supplement.
ISSN: 0941-018X. ISBN: 3-437-11362-3, 1-56081-333-4.
DOCUMENT TYPE: Book
Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 16 Jul 1992
Last Updated on STN: 16 Jul 1992

L11 ANSWER 63 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 26

ACCESSION NUMBER: 1992-13545 BIOTECHDS
TITLE: High level **expression** of **streptokinase** in Escherichia coli;
gene **cloning**, **expression** and purification of thrombolytic protein
AUTHOR: Estrada M P; Hernandez L; Perez A; Rodriguez P; *de la Fuente J; Herrera L
LOCATION: Mammalian Cell Genetics Division, Centro de Ingenieria Genetica y Biotecnologia, P.O. Box 6162, Havana 6, Cuba.
SOURCE: Bio/Technology; (1992) 10, 10, 1138-42
CODEN: BTCHDA
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The **streptokinase** (SK) gene was isolated by the polymerase chain reaction from Streptomyces **equisimilis** ATCC 9542. The

5-amplification primer introduced an ATG codon for translation initiation in Escherichia coli. The amplified fragment, which lacked the signal peptide sequence, was digested with BamHI, inserted into vector plasmid pTrp (to obtain plasmid pEKG-3 containing the SK gene under the control of a trp promoter), and used to transform E. coli HB101 cells. The DNA sequence of the SK gene region contained 5 differences at the amino acid level with respect to the reported SK protein. Plasmid pEKG-3 was introduced into E. coli K-12 strain W3110 for **expression**. The trp promoter was induced, and maximal SK **expression** was obtained after 14 hr, at which time the plasmid copy number reached 420 copies/cell. The **recombinant** SK was found in the cell cytosol and constituted 25% of total cell protein. It was purified by affinity chromatography using acylated human plasminogen coupled to Sepharose-4B, and ionexchange chromatography on DEAE-Sephacel. The **recombinant** product and natural SK had equivalent biological activities. (38 ref)

L11 ANSWER 64 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1992:646505 HCAPLUS
DOCUMENT NUMBER: 117:246505
TITLE: **Streptokinase** mutation affecting **skc** **expression** in homologous and heterologous hosts
AUTHOR(S): Mechold, U.; Muller, J.; Malke, H.
CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Ther., Jena, D-6900, Germany
SOURCE: Zentralblatt fuer Bakteriologie, Supplement (1992), 22(New Perspect. Streptococci Streptococcal Infect.), 336-8
CODEN: ZBASE2; ISSN: 0941-018X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Mutations affecting the level of **streptokinase** gene **skc** **expression** and/or secretion in homologous and heterologous hosts are phys. characterized. The principal classes of mutations produced included **skc** deletions, IS element insertions, and **skc** duplications. The deletion events, represented by mutations Δ(**skc**)-247 and Δ(**skc**)-305 present in plasmids pMM247 and pMM305, resp., removed a tetrapeptide (F10-L13 or L12-A15) from the hydrophobic core of the **Skc** signal sequence. These mutations, reduced the size, hydrophobicity and predicted alpha-helicity of the central region of the signal sequence. The corresponding plasmids, upon transformation into E. coli and P. mirabilis L-forms, substantially increased the level of **Skc** **expression** in either host. In E. coli, they also facilitated the export of mature **Skc** into the culture medium. In the gram-pos. hosts, **skc** **expression** was less dramatically affected; however, the proportion of **Skc** activity found in the culture medium was significantly decreased when compared to the extracellular activity resulting from wild type **skc**. IS1 insertion did not alter the primary structure of the promoter but displaced in upward direction, by 768 bp, a static DNA bending locus having its center some 140 bp upstream of the -35 region in wild type DNA. When studied with plasmid pMM697, this insertion event resulted in severely decreased **Skc** **expression** in all hosts but, expectedly, did not affect **Skc** secretability. Gene **skc** duplication in the chromosome of the homologous producer strain, S. **equisimilis** H46A, was achieved by a single crossover event between the chromosomes and an integrateable **Skc** plasmid, pSM752, in the region of shared homol. As judged by Southern hybridization, cells transiently supporting the replication of pSM752 gave rise to a stable erythromycin-resistant **clone** designated H46SM which was plasmid-free and produced **Skc** at levels approx. twice as high as the wild type.

L11 ANSWER 65 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:585755 HCAPLUS
DOCUMENT NUMBER: 117:185755
TITLE: High-level **expression** of degraded product-free **streptokinase** in Escherichia coli by removal of its putative leader sequence. [Erratum to document cited in CA116(13):122160m]
AUTHOR(S): Park, Seung Kook; Jang, Jeong Su; Kim, Jee Cheon; Chun, Moon Jin; Byun, Si Myung
CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol., Seoul, 130-650, S. Korea
SOURCE: Molecules and Cells (1992), 2(1), 119
CODEN: MOCEEK; ISSN: 1016-8478
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The omission of acknowledgment of a research grant has been corrected The error was not reflected in the abstract or the index entries.

L11 ANSWER 66 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1992-03808 BIOTECHDS
TITLE: Method for the isolation and **expression** of a gene encoding **streptokinase**; Streptococcus **equisimilis** gene **cloning** and vector plasmid pEKG3, plasmid pPESKC-4 and plasmid pPISKC-6 **expression** in Escherichia coli or Pichia pastoris
PATENT ASSIGNEE: Cent.Ing.Genet.Biotecnol.
PATENT INFO: AU 9178101 28 Nov 1991
APPLICATION INFO: AU 1991-78101 31 May 1991
PRIORITY INFO: CU 1990-90 23 May 1990
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1992-024716 [04]
AB A new method for the isolation and **expression** of a gene, **SKC-2**, encoding Streptococcus **equisimilis** C (ATCC 9542) **streptokinase** comprises (i) gene amplification from synthetic oligonucleotides SK1, SK2 and SK3 (specified DNA sequence), (ii) **cloning SKC-2** in bacteria (preferably Escherichia coli) with or without a signal peptide; and (iii) intra- or extracellular **expression** in yeast (preferably Pichia pastoris), with the transformed microorganism displaying a high stability and level of **expression**. The following are also claimed: (1) plasmid pEKG3 containing **SKC-2** inserted between the trp promoter and the phage T4 terminator for **expression** in bacteria; (2) plasmid pPESKC-4 and plasmid pPISKC-6, obtained by insertion of **SKC-2** in the yeast **expression** vectors plasmid pPS-7 and plasmid pNAO, respectively, for extra- or intracellular **expression**; (3) transformed microorganisms displaying high levels of **SKC-2** gene **expression**, good viability and cellular stability; (4) the product resulting from **expression** of the **SKC-2** gene in bacteria and yeast; (5) **recombinant** DNA comprising the **SKC-2** DNA sequence; and (6) the **expression** product of the **recombinant** DNA. (28pp)

L11 ANSWER 67 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:672660 HCAPLUS
DOCUMENT NUMBER: 115:272660
TITLE: **Recombinant** of thrombolytic and fibrinolytic enzymes as inactive dimers linked by sequence recognized by blood coagluation factors
INVENTOR(S): Dawson, Keith Martyn; Hunter, Michael George; Czaplewski, Lloyd George
PATENT ASSIGNEE(S): British Bio-Technology Ltd., UK
SOURCE: PCT Int. Appl., 110 pp.

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9109125	A1	19910627	WO 1990-GB1911	19901207
W: AU, CA, FI, HU, JP, KR, NO, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
CA 2069085	AA	19910608	CA 1990-2069085	19901207
CA 2069085	C	20000201		
CA 2069105	AA	19910608	CA 1990-2069105	19901207
AU 9169540	A1	19910718	AU 1991-69540	19901207
AU 644399	B2	19931209		
ZA 9009853	A	19920826	ZA 1990-9853	19901207
ZA 9009854	A	19920826	ZA 1990-9854	19901207
EP 504241	A1	19920923	EP 1991-900869	19901207
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 155812	E	19970815	AT 1991-900851	19901207
ES 2106073	T3	19971101	ES 1991-900851	19901207
IL 96601	A1	19990509	IL 1990-96601	19901207
JP 05502374	T2	19930428	JP 1991-501314	19911115
JP 2900606	B2	19990602		
US 5434073	A	19950718	US 1992-854596	19920603
FI 9202609	A	19920605	FI 1992-2609	19920605
NO 9202237	A	19920806	NO 1992-2237	19920605
AU 9344976	A1	19931118	AU 1993-44976	19930830

PRIORITY APPLN. INFO.: GB 1989-27722 A 19891207
WO 1990-GB1911 A 19901207

AB Fibrinolytic or thrombolytic enzymes are manufactured in a **recombinant** host as inactive fusion proteins containing two or more sequences of the protein linked by a peptide that can be cleaved by a blood-coagulation factor. The construction of **expression** vectors for the manufacture of hirudin or **streptokinase** dimers linked by peptides cleavable by Factor Xa or thrombin for Escherichia col or Saccharomyces cerevisiae (with or without product secretion) is described. All the products tested were cleavable by the appropriate factors.

L11 ANSWER 68 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:443480 HCAPLUS
DOCUMENT NUMBER: 115:43480
TITLE: Synthetic genes for **streptokinase** and **streptokinase** analogs and their **expression** in Escherichia coli
INVENTOR(S): Fujii, Setsuro; Katano, Tamiki; Majima, Eiji; Ogino, Koichi; Ono, Kenji; Sakata, Yasuyo; Uenoyama, Tsutomu
PATENT ASSIGNEE(S): Otsuka Pharmaceutical Factory, Inc., Japan
SOURCE: Eur. Pat. Appl., 76 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 407942	A2	19910116	EP 1990-113099	19900709
EP 407942	A3	19910904		
EP 407942	B1	19951011		
R: AT, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE				
JP 04011892	A2	19920116	JP 1990-179851	19900706
US 5240845	A	19930831	US 1990-549049	19900706

AU 9058806	A1	19910117	AU 1990-58806		19900709
AU 648029	B2	19940414			
AT 129014	E	19951015	AT 1990-113099		19900709
ES 2078925	T3	19960101	ES 1990-113099		19900709
CA 2020828	AA	19910112	CA 1990-2020828		19900710
PRIORITY APPLN. INFO.:			JP 1989-179432	A	19890711
			JP 1989-307957	A	19891127
			JP 1990-96830	A	19900411

AB Genes encoding **streptokinase** (I) and its derivs. are synthesized and **expressed** in a host such as Escherichia coli for manufacture of I suitable for clin. application. The DNA encoding natural-type I was synthesized by standard chemical and used for construction of **expression** plasmid pSKXT, which in turn **expressed** the I gene using the E. coli tac promoter and the blc signal sequence. Efficient **expression** of the gene in the E. coli transformants and purification of the protein product were demonstrated. I analogs with a carboxy-terminal deletions, optionally with internal modifications were also described.

L11 ANSWER 69 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1991:513401 BIOSIS
DOCUMENT NUMBER: PREV199141114116; BR41:114116
TITLE: **EXPRESSION** AND PROPERTIES OF HYBRID **STREPTOKINASES** EXTENDED BY AMINO-TERMINAL PLASMINOGEN KRINGLE DOMAINS.
AUTHOR(S): MALKE H [Reprint author]; FERRETTI J J
CORPORATE SOURCE: DEP MICROBIOL IMMUNOL, UNIV OKLA HEALTH SCI CENTER, OKLAHOMA CITY, OKLA 73190, USA
SOURCE: (1991) pp. 184-189. DUNNY, G. M., P. P. CLEARY AND L. L. MCKAY (ED.). GENETICS AND MOLECULAR BIOLOGY OF STREPTOCOCCI, LACTOCOCCI, AND ENTEROCOCCI; THIRD INTERNATIONAL ASM (AMERICAN SOCIETY FOR MICROBIOLOGY) CONFERENCE, MINNEAPOLIS, MINNESOTA, USA, JUNE 6-9, 1990. VIII+310P. AMERICAN SOCIETY FOR MICROBIOLOGY: WASHINGTON, D.C., USA. ILLUS.
ISBN: 1-55581-034-9.
DOCUMENT TYPE: Book
Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 14 Nov 1991
Last Updated on STN: 14 Nov 1991

L11 ANSWER 70 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:100297 HCAPLUS
DOCUMENT NUMBER: 116:100297
TITLE: Secretion of **streptokinase** from Bacillus subtilis
AUTHOR(S): Kim, Sung Il; Lee, Se Yong; Byun, Si Myung
CORPORATE SOURCE: Dep. Agric. Chem., Korea Univ., Seoul, 136-701, S. Korea
SOURCE: Molecules and Cells (1991), 1(3), 325-31
CODEN: MOCEEK; ISSN: 1016-8478
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To secret the **streptokinase** (**SKC**) from B. subtilis, three different **expression**-secretion vectors were constructed. One of them was pB140SK-I which contained the full **streptokinase** gene (**skc**) of Streptococcus **equisimilis** ATCC 9542 on pUB140 plasmid and the others were pB240SK-II and pB340SK-II which possessed the structural gene part of **skc** on secretion vectors pUBA240 and pUBS340, resp. PUBA240 and pUBS340 are secretion vectors constructed with α-amylase promoter (amyR2) and signal sequence. When these **streptokinase** secretion vectors were transferred into

B. subtilis LKS86, **streptokinase** was **expressed** and secreted into the culture broth. Although own promoter and signal sequence of the **skc** gene functioned normally in B. subtilis, maximum amount of **streptokinase** secretion was obtained by LKS86(pB240SK-II) system.

L11 ANSWER 71 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 27
ACCESSION NUMBER: 1991:96194 HCAPLUS
DOCUMENT NUMBER: 114:96194
TITLE: The leader sequence of **streptokinase** is responsible for its post-translational carboxyl-terminal cleavage
AUTHOR(S): Park, Seung Kook; Lee, Byeong Ryong; Byun, Si Myung
CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol., Seoul, 130-650, S. Korea
SOURCE: Biochemical and Biophysical Research Communications (1991), 174(1), 282-6
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB When the **streptokinase** gene from Streptococcus equisimillis was **expressed** from 2 tac promoter-controlled **expression** vectors, one deleted the putative leader sequence of **streptokinase**. Both normal and degraded **streptokinase** were detected in proteins **expressed** from the leader-encoding vector, but only normal **streptokinase** was detected from the leader-deleted vector. These findings indicate that the characteristic carboxyl-terminal cleavage of **streptokinase** is correlated with its leader sequence and occurs during defective secretion. A homogeneous preparation of **streptokinase** was facilitated by **expression** from this leader-deleted vector.

L11 ANSWER 72 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1992:122160 HCAPLUS
DOCUMENT NUMBER: 116:122160
TITLE: High-level **expression** of degraded product-free **streptokinase** in Escherichia coli by removal of its putative leader sequence
AUTHOR(S): Park, Seung Kook; Jang, Jeong Su; Kim, Jee Cheon; Chun, Moon Jin; Byun, Si Myung
CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol., Seoul, 130-650, S. Korea
SOURCE: Molecules and Cells (1991), 1(2), 187-92
CODEN: MOCEEK; ISSN: 1016-8478
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **expression** vector for **streptokinase** has been constructed from the previously **cloned streptokinase**-coding gene (**skc**) from Streptococcus **equisimilis**. Because of its deleterious effect on the host cell growth, the leader sequence of **skc** was removed and the leader sequence-deleted **skc** was subcloned into the vector pKK223-3, which contains the regulatable tac promoter and rrnB T1T2 transcription terminator, with a short synthetic oligonucleotide adapter. When this vector, pKS601 having **skc** gene, was **expressed** in E. coli, a 47.4-kDa protein was found to be newly accumulated to about 12% of the total cellular proteins, and it was identified as the **streptokinase** by immunoblotting with rabbit anti-**streptokinase** polyclonal serum. The **expressed streptokinase** was free from carboxyl-terminal degraded 44-kDa **streptokinase** and purified to near homogeneity using DEAE-cellulose and Sephadex G-150 columns. Its specific activity was about 1.3 + 105 CLN units/mg protein.

L11 ANSWER 73 OF 106 MEDLINE on STN DUPLICATE 28

ACCESSION NUMBER: 92039051 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1937032
TITLE: Isolation, sequence and **expression** in Escherichia coli, Bacillus subtilis and Lactococcus lactis of the DNase (streptodornase)-encoding gene from Streptococcus **equisimilis** H46A.
AUTHOR: Wolinowska R; Ceglowski P; Kok J; Venema G
CORPORATE SOURCE: Department of Pharmaceutical Microbiology, Medical Academy, Warsaw, Poland.
SOURCE: Gene, (1991 Sep 30) 106 (1) 115-9.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M59725; GENBANK-M59726; GENBANK-M59727; GENBANK-M59728; GENBANK-M63990; GENBANK-S61507; GENBANK-S63856; GENBANK-S63863; GENBANK-S65020; GENBANK-S65060; GENBANK-X17241
ENTRY MONTH: 199112
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19920124
Entered Medline: 19911223

AB A partial library of BclI-generated chromosomal DNA fragments from Streptococcus **equisimilis** H64A (Lancefield Group C) was constructed in Escherichia coli. **Clones** displaying either **streptokinase** or deoxyribonuclease (streptodornase; SDC) activities were isolated. The gene (sdc) **expressing** the SDC activity was allocated on the 1.1-kb AccI DNA subfragment. Sequence analysis of this DNA fragment revealed the presence of one open reading frame, which could encode a protein of 36.8 kDa. The N-terminal portion of the deduced protein exhibited features characteristic of prokaryotic signal peptides. The sdc gene was **expressed** in E. coli, Bacillus subtilis and Lactococcus lactis. As observed for S. **equisimilis**, in the heterologous Gram + hosts, at least part of the SDC protein was secreted into the medium.

L11 ANSWER 74 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 29
ACCESSION NUMBER: 1991-08049 BIOTECHDS
TITLE: High yield production of **recombinant** serotype-C **streptokinase**;
gene **cloning**; **expression** in Streptococcus **equisimilis** containing 2 gene copies and a selectable marker gene using vector plasmid pSM752 or plasmid pSMS69
PATENT ASSIGNEE: Akad.Wiss.DDR
PATENT INFO: DD 284898 28 Nov 1990
APPLICATION INFO: DD 1989-332866 21 Sep 1989
PRIORITY INFO: DD 1989-332866 21 Sep 1989
DOCUMENT TYPE: Patent
LANGUAGE: German
OTHER SOURCE: WPI: 1991-125491 [18]

AB **Recombinant** serotype-C **streptokinase** (**Skc**) production comprises cultivation of Streptococcus **equisimilis** H464, C9/50 or SSIC type 20 transformants harboring a vector plasmid that includes the **Skc** gene and a selectable marker (SM) gene as well as **expression** and protein secretion signals. The transformant is cultured for 8-20 generations without selection pressure, and strains lacking plasmids but carrying the specified markers are then cultured for **Skc** production. The SM gene preferably encodes resistance to macrolide-lincosamide-streptogramin B (MLS), chloramphenicol, kanamycin or streptomycin. The transformants contain 2 copies of the **Skc** gene incorporated into their chromosomes by homologous recombination.

They produce **Skc** at a greater rate than e.g. H46A. The inherited marker is retained stably over many generations. The **Skc** gene is preferably present on a PstI fragment (transformation is then with plasmid pSM752) or a 2076 bp fragment (transformation with plasmid pSMS69); both vectors contain the MLS-resistance marker. Transformation is by electroporation or PEG-mediated protoplast transformation. (12pp)

L11 ANSWER 75 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1991-01878 BIOTECHDS
TITLE: Constructing vector for detecting **expression** of foreign genes;
by inserting element containing **expression** unit, **streptokinase** gene and restriction sites, allowing in frame gene insertion; pro-chymosin, beta-galactosidase production
PATENT ASSIGNEE: Akad.Wiss.DDR
PATENT INFO: DD 279900 20 Jun 1990
APPLICATION INFO: DD 1987-306609 3 Sep 1987
PRIORITY INFO: DD 1987-306609 3 Sep 1987
DOCUMENT TYPE: Patent
LANGUAGE: German
OTHER SOURCE: WPI: 1990-342373 [46]
AB Construction of vectors for detecting heterologous gene **expression** comprises: i. incorporating (in order from N terminus) into a **cloning** vector, a polylinker or restriction site (RS1); **expression** or **expression** secretion unit (Escherichia coli lac operon or exotoxin A gene of phage T12 from Streptococcus pyogenes; polylinker or restriction site (RS2); **streptokinase** (SK) structural gene (from Streptococcus **equisimilis** H46A, particularly a 1596 bp HindIII fragment from plasmid pMF5), a polylinker or restriction site (RS3); ii. inserting a foreign gene, X, without a promoter into polylinker or RS2; iii. the resulting detection vector, encoding for an X-SK fusion product, is used to transform microbial receptor cells; and iv. subjecting **recombinant clones** to a plasminogen-milk agar (PMA) overlaying test. Preferably, the vector is a bacterial plasmid or an M13 E. coli phage vector. X is a pro-chymosin gene (plasmid pHRW400 or plasmid pHRW500), human interferon-alpha-1, or beta-galactosidase (EC-3.2.1.23, from a pUC plasmid). Recipients are E. coli JM101, Streptococcus sanguis Challis 6 or Streptococcus lactis MG1363. (14pp)

L11 ANSWER 76 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:179754 HCAPLUS
DOCUMENT NUMBER: 114:179754
TITLE: Fusion proteins of **streptokinase** and human plasminogen
INVENTOR(S): Malke, Horst; Ferretti, Joseph J.
PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Zentralinstitut fuer Mikrobiologie und Experimentelle Therapie, Ger. Dem. Rep.
SOURCE: Ger. (East), 42 pp.
CODEN: GEXXA8
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 284484	A5	19901114	DD 1989-328631	19890516
PRIORITY APPLN. INFO.:			DD 1989-328631	19890516

AB A hybrid **streptokinase** is produced in prokaryotic cells. The **streptokinase**, which displays thrombin selectivity, consists of

the N-terminal kringle domains of human plasminogen fused to C-terminal Streptococcus **equisimilis** **streptokinase**. Escherichia coli transformed with plasmids encoding the described fusion protein fused to the N-terminal hexapeptide of β-galactosidase produced the hybrid **streptokinase** which was purified from cell lysates by immuno-affinity chromatog. and by chromatog. on lysine Sepharose.

L11 ANSWER 77 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:162447 HCAPLUS
DOCUMENT NUMBER: 114:162447
TITLE: **Recombinant** manufacture of **streptokinase**
INVENTOR(S): Laplace, Frank; Mueller, Joerg; Malke, Horst
PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Patentabteilung, Ger. Dem. Rep.
SOURCE: Ger. (East), 9 pp.
CODEN: GEXXA8
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 282709	A5	19900919	DD 1988-323948	19881227

PRIORITY APPLN. INFO.: DD 1988-323948 19881227
AB **Streptokinase** from Streptococcus **equisimilis** serotype C is manufactured by **expression** of the **skc** gene in Escherichia coli, Bacillus subtilis, or other Streptococcus. The natural **expression** cassette for the **skc** gene was introduced into a broad host-range vector to give plasmid pMLS10. Transformants of Streptococcus sanguis carrying this vector produced 750-1,000 **streptokinase** units/mL in a complex medium after 16 h growth at 36°, at this point the culture reached stationary phase and the enzyme continued to be slowly accumulated.

L11 ANSWER 78 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:141637 HCAPLUS
DOCUMENT NUMBER: 114:141637
TITLE: Manufacture of **streptokinase** with bacterial L-forms
INVENTOR(S): Laplace, Frank; Mueller, Joerg; Gumpert, Johannes; Malke, Horst
PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Patentabteilung, Ger. Dem. Rep.
SOURCE: Ger. (East), 7 pp.
CODEN: GEXXA8
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 281816	A5	19900822	DD 1988-323947	19881227

PRIORITY APPLN. INFO.: DD 1988-323947 19881227
AB **Streptokinase** is manufactured by **expression** of the **skc** gene from serotype C of Streptococcus **equisimilis** in the L-form of a bacterial host (Bacillus or Proteus). The construction of the small plasmid pMLS10 (6.7 kb) for **expression** of the gene in L-forms of P. mirabilis and B. subtilis is described.

L11 ANSWER 79 OF 106 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN DUPLICATE 30

ACCESSION NUMBER: 90356604 EMBASE
DOCUMENT NUMBER: 1990356604
TITLE: Duplication of the **streptokinase** gene in the chromosome of Streptococcus **equisimilis** H46A.
AUTHOR: Muller J.; Malke H.
CORPORATE SOURCE: Central Inst. Microbiology, Beutenbergstrasse 11,Jena-6900, Germany
SOURCE: FEMS Microbiology Letters, (1990) Vol. 72, No. 1-2, pp. 75-78.
ISSN: 0378-1097 CODEN: FMLED7
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 911213
Last Updated on STN: 911213

AB The erythromycin resistance plasmid pSM752 carrying the **cloned** **streptokinase** gene, **skc**, was introduced by protoplast transformation into Streptococcus **equisimilis** H46A from which **skc** was originally **cloned**. Cells transiently supporting the replication of pSM752 gave rise to an erythromycin-resistant **clone** designated H46SM which was plasmid free and produced **streptokinase** at levels approximately twice as high as the wild type. Southern hybridization of total cell DNA with an **skc** -containing probe provided evidence for the duplication of the **skc** gene in the H46SM chromosome. The results, which have some bearing on industrial **streptokinase** production, can be best explained by a single cross-over event between the chromosome and the plasmid in the region of shared homology leading to the integration of pSM752 in a Campbell-like manner.

L11 ANSWER 80 OF 106 MEDLINE on STN
ACCESSION NUMBER: 91130849 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2283044
TITLE: Duplication of the **streptokinase** gene in the chromosome of Streptococcus **equisimilis** H46A.
AUTHOR: Muller J; Malke H
CORPORATE SOURCE: Academy of Sciences of the G.D.R., Central Institute of Microbiology and Experimental Therapy, Jena.
SOURCE: FEMS microbiology letters, (1990 Oct) 60 (1-2) 75-8.
Journal code: 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199103
ENTRY DATE: Entered STN: 19910405
Last Updated on STN: 19970203
Entered Medline: 19910321

AB The erythromycin resistance plasmid pSM752 carrying the **cloned** **streptokinase** gene, **skc**, was introduced by protoplast transformation into Streptococcus **equisimilis** H46A from which **skc** was originally **cloned**. Cells transiently supporting the replication of pSM752 gave rise to an erythromycin-resistant **clone** designated H46SM which was plasmid free and produced **streptokinase** at levels approximately twice as high as the wild type. Southern hybridization of total cell DNA with an **skc** -containing probe provided evidence for the duplication of the **skc** gene in the H46SM chromosome. The results, which have some bearing on industrial **streptokinase** production, can be best explained by a single cross-over event between the chromosome and the plasmid in the region of shared homology leading to the integration of pSM752 in a Campbell-like manner.

L11 ANSWER 81 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1989:206803 HCAPLUS
DOCUMENT NUMBER: 110:206803
TITLE: **Streptokinase** mutations relieving Escherichia coli K-12 (prlA4) of detriments caused by the wild-type **skc** gene
AUTHOR(S): Mueller, Joerg; Reinert, Hilmer; Malke, Horst
CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Therapy, Acad. Sci. G. D. R., Jena, 6900, Ger. Dem. Rep.
SOURCE: Journal of Bacteriology (1989), 171(4), 2202-8
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A novel phenotype is described for E. coli K-12 carrying the prlA4 allele determining a membrane component of the protein export mechanism. It is manifest as transformation deficiency for plasmids containing the **cloned** group C streptococcal **streptokinase** gene, **skc**. **Streptokinase** plasmid mutations relieving the prlA4 strain of this deficiency fell into three classes. Class 1 included **skc**::IS5 insertions, with IS5 integrated in a region encoding the **Skc** signal sequence and inactivating **skc**. Class 2 included IS1 insertions leaving **skc** intact but reducing **skc expression**, presumably by altering the function of the **skc** promoter as judged by an insertion site close to the -35 region. Class 3 included **skc** deletions removing the entire signal sequence or a tetrapeptide from its hydrophobic core. The tetrapeptide deletion reduced the size, hydrophobicity, and predicted α-helicity of the central region of the **Skc** signal sequence but facilitated the export of mature **Skc** in both the wild type and the prlA4 mutant. These findings indicate that the incompatibility between prlA4 and **skc** is related to deleterious effects of the **Skc** signal sequence. The tetrapeptide deletion may function by altering the conformation of the signal sequence so as to render interaction with both the PrlA wild-type protein and the PrlA4 mutant protein less detrimental to the export mechanism. These findings also provide an explanation for the difficulties encountered in **cloning streptokinase** genes in E. coli plasmids and maintaining their structural stability.

L11 ANSWER 82 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1990-02600 BIOTECHDS
TITLE: Site-specific alteration of Gly-24 in **streptokinase** : its effect on plasminogen activation; site-directed mutagenesis effect on plasminogen-activator activity; gene **cloning** and **expression** in Escherichia coli
AUTHOR: Lee B R; Park S K; Kim J H; *Byun S M
LOCATION: Department of Biological Science and Engineering, Korea Advanced Institute of Science and Technology (KAIST), P.O. Box 150, Cheongryang, Seoul, Korea.
SOURCE: Biochem.Biophys.Res.Commun.; (1989) 165, 3, 1085-90
CODEN: BBRCA9
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Oligonucleotide site-directed mutagenesis was performed to replace Gly-24 of Streptococcus **equisimilis** (ATCC 9542) **streptokinase** with His, Glu, or Ala. The **streptokinase** gene was **cloned**, subjected to mutagenesis for removal of the RsaI site, **cloned** into vector plasmid pKS601 under the control of the trp promoter and used to transform Escherichia coli C600. The **recombinant** proteins were purified by DEAE-cellulose and Sephadex-G150 chromatography. Substitutions with either His or Glu gave almost complete loss of **streptokinase** activity but

streptokinase replaced with Ala retained its activity. Although **streptokinases** with His-24 or Glu-24 bound normally to human plasminogen, they did not generate active plasmin, whereas those with Ala-24 or Gly-24 generated active plasmin. The results indicate that the small, uncharged alkyl group side chain on the 24th amino acid residue of **streptokinase** is indispensable for the activity of the human plasminogen-**streptokinase** complex. A charged amino acid in position 24 disrupts beta-sheet formation and prevents **streptokinase** from adopting the orientation required for plasminogen activation. (26 ref)

L11 ANSWER 83 OF 106 MEDLINE on STN DUPLICATE 31
ACCESSION NUMBER: 90172183 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2625666
TITLE: Sequence-directed DNA bending upstream of the **streptokinase** promoter.
AUTHOR: Muller J; Malke H
CORPORATE SOURCE: Akademie der Wissenschaften der DDR.
SOURCE: Journal of basic microbiology, (1989) 29 (9) 611-6.
Journal code: 8503885. ISSN: 0233-111X.
PUB. COUNTRY: GERMANY, EAST: German Democratic Republic
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199004
ENTRY DATE: Entered STN: 19900601
Last Updated on STN: 19900601
Entered Medline: 19900409

AB A 450-base pair (bp) HinfI restriction fragment from the chromosome of Streptococcus **equisimilis** H46A contains the early coding region of the **streptokinase** gene (**skc**), the **skc** promoter, and a stretch of DNA 5' to the--35 region of the **skc** promoter. Two-dimensional polyacrylamide (PA) gel electrophoresis at two different temperatures showed that this fragment migrates anomalously slowly on PA gels, suggesting the existence of a bent DNA conformation. Inspection of the nucleotide sequence confirmed this suggestion by revealing numerous oligomeric dA.dT tracts, some of which are in phase with the helix screw. Computer analysis of the sequence predicted the existence of two bending loci, one of which is located upstream of the **skc** promoter. In addition to showing DNA bending, the 450-bp HinfI fragment contains multiple 13-bp sequences homologous to the Escherichia coli integration host factor DNA-binding consensus sequence. Insertion of IS1 into a site immediately upstream of the--35 region decreased the **expression** level of **skc** in E. coli, suggesting that DNA conformation upstream of the promoter has a role in **skc expression**.

L11 ANSWER 84 OF 106 MEDLINE on STN DUPLICATE 32
ACCESSION NUMBER: 88302119 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3043172
TITLE: Tripartite **streptokinase** gene fusion vectors for gram-positive and gram-negative procaryotes.
AUTHOR: Klessen C; Schmidt K H; Ferretti J J; Malke H
CORPORATE SOURCE: Academy of Sciences of the GDR, Central Institute of Microbiology and Experimental Therapy, Jena.
SOURCE: Molecular & general genetics : MGG, (1988 May) 212 (2) 295-300.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198809
ENTRY DATE: Entered STN: 19900308

Last Updated on STN: 19900308
Entered Medline: 19880921

AB A specific 1,596 bp HincII fragment ('**skc**) from the chromosome of Streptococcus **equisimilis** contains an active **streptokinase** (SK) gene (**skc**) lacking, in addition to the **expression** signals, codons 1 through 39 of wild-type **skc** but retaining the remainder of the **skc** coding sequence together with the transcription terminator. Using this fragment as an indicator gene, we constructed two types of vectors which in appropriate hosts resulted in the synthesis of SK fusion proteins after insertional activation of '**skc**. The first type are open reading frame (ORF) vectors in which '**skc** was inserted into pUC18 out of frame with respect to lacZ', thus conferring an SK-negative phenotype. Any DNA fragments representing ORFs inserted between the lacZ' **expression** signals and '**skc** such that the **skc** reading frame was restored resulted in the production of tripartite proteins which exhibited SK activity. The second type of vector, which functioned in both gram-positive and gram-negative bacteria, used the streptococcal speA **expression** and secretion signals in front of the ORF to activate '**skc** insertionally. Using a large fragment from the chymosin gene as the target sequence, the usefulness of these vectors for studying foreign gene **expression** in streptococci as well as Escherichia coli was demonstrated.

L11 ANSWER 85 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1988-10064 BIOTECHDS
TITLE: **Expression** of heterologous genes in Pseudomonas putida under the control of streptococcal promoters; interferon-alpHA-1 and **streptokinase** gene **expression**
AUTHOR: Laplace F; Mueller J; Wollweber L; Malke H
LOCATION: Central Institute of Microbiology and Experimental Therapy, Beutenbergstrasse 11, Jena 6900, DDR.
SOURCE: FEMS Microbiol.Lett.; (1988) 52, 275-78
CODEN: FMLED7
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Expression** plasmids were constructed for Pseudomonas putida KT2440 using streptococcal promoters. The speA promoter/ interferon-alpha1 (IFN-alpha1) cassette from plasmid pJC127 was inserted in plasmid pBR322, and joined with RSF1010 to form plasmid pJP127. The **streptokinase** (SK) gene (**skc**) and promoter from plasmid pMF5 were inserted in plasmid pUC19 and fused with RSF1010 to form plasmid pLMM192. **Skc** was **expressed** in P. putida with pLMM192, reaching maximum SK yields in early stationary phase (0.2-0.4 ug/ml culture). Most activity was distributed equally between periplasm and cytoplasm, with 2-5% in the supernatant. SDS-PAGE and protease treatment showed the P. putida protein was identical to those of Streptococcus **equisimilis** H46A and Streptococcus sanguis Challis. P. putida with pJP127 produced 100 000 U/l interferon (determined by radial immunodiffusion and ELISA). Immunodiffusion showed the protein to be identical with human IFN-alpha1. Antiviral assays showed that 70% activity was cytoplasmic, the remainder being periplasmic. Thus neither secretion vector mediated complete export into the periplasm. (29 ref)

L11 ANSWER 86 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 33
ACCESSION NUMBER: 1988-02891 BIOTECHDS
TITLE: **recombinant streptokinase** production in yeasts; plasmid vector **cloning** in Pichia sp. for thrombolytic enzyme preparaton
PATENT ASSIGNEE: Phillips-Petrol.

PATENT INFO: EP 248227 9 Dec 1987
APPLICATION INFO: EP 1987-106614 7 May 1987
PRIORITY INFO: US 1986-960 8 May 1986
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1987-343212 [49]

AB The production of **recombinant streptokinase** for use in thrombolytic enzyme preparations is new. A novel DNA sequence comprises: a yeast regulatory region which is capable of controlling transcription of mRNA in yeast when positioned at the 5'-end of a polypeptide encoding region; and a polypeptide coding region encoding **streptokinase** or portions thereof free from bacterial signal sequence. The regulatory region (RR) is preferably selected from the Pichia AOX1 RR, DAS1 RR, Pichia glyceraldehyde-3-phosphate-dehydrogenase RR, Saccharomyces cerevisiae acid phosphatase RR, Saccharomyces alpha-mating factor RR, Saccharomyces glyceraldehyde-5-phosphate-dehydrogenase RR and the Pich p40 RR. Also new is the DNA sequence contained in a vector, plasmid pHTskc25, along with a selectable marker gene and a yeast autonomous replication sequence. **Streptokinase** may be produced at high yields by culturing Pichia sp. transformed with plasmid pHTskc25. The **streptokinase** gene is from Streptococcus **equisimilis**. (27pp)

L11 ANSWER 87 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1987:499919 BIOSIS
DOCUMENT NUMBER: PREV198733127633; BR33:127633
TITLE: **STREPTOKINASE EXPRESSION** OF ALTERED FORMS.
AUTHOR(S): MALKE H [Reprint author]; LORENZ D; FERRETTI J J
CORPORATE SOURCE: ACAD SCI GER DEMOCRATIC REPUBLIC, CENT INST MICROBIOL AND EXP THERAPY, DDR-69 JENA, GDR
SOURCE: (1987) pp. 143-149. FERRETTI, J. J. AND R. CURTISS, III (ED.). STREPTOCOCCAL GENETICS; SECOND ASM (AMERICAN SOCIETY FOR MICROBIOLOGY) CONFERENCE, MIAMI, FLORIDA, USA, MAY 21-24, 1986. VIII+300P. AMERICAN SOCIETY FOR MICROBIOLOGY: WASHINGTON, D.C., USA. ILLUS.
ISBN: 0-914826-93-X.
DOCUMENT TYPE: Book
Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 27 Nov 1987
Last Updated on STN: 27 Nov 1987

L11 ANSWER 88 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1988:487410 HCAPLUS
DOCUMENT NUMBER: 109:87410
TITLE: Methylotrophic yeast as vehicles for heterologous gene **expression**
AUTHOR(S): Stroman, D. W.; Hagenson, M. J.
CORPORATE SOURCE: Phillips Res. Cent., Phillips Pet. Co., OK, USA
SOURCE: DECHEMA Monographien (1987), 105(Physiol. Genet. Modulation Prod. Form.), 141-6
CODEN: DMDGAG; ISSN: 0070-315X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The methylotrophic yeast, Pichia pastoris, has been developed as a superior **recombinant** DNA (rDNA) production host. The key component in the development of this host was the **cloning** of the alc. oxidase gene and use of its promoter-regulatory region to control gene **expression**. Heterologous **expression** of several foreign genes in this yeast has been studied. The promoter-regulatory region from the alc. oxidase gene permits very high per cell levels of gene

expression in an easily regulated manner. These high per cell levels of **expression** can be combined with high cell d. fermentation technol. to yield very high per L production of rDNA products. This is shown by the high levels of production of **streptokinase** in this yeast.

L11 ANSWER 89 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1986:455636 HCAPLUS
DOCUMENT NUMBER: 105:55636
TITLE: The **streptokinase** gene: **cloning**, sequencing and **expression** in new hosts
AUTHOR(S): Malke, Horst
CORPORATE SOURCE: Zentralinst. Mikrobiol., Dtsch. Akad. Wiss., Jena, Ger. Dem. Rep.
SOURCE: Zeitschrift fuer Klinische Medizin (1985) (1986), 41(7), 502-4
CODEN: ZKMEEF; ISSN: 0233-1608
DOCUMENT TYPE: Journal
LANGUAGE: German
AB The **streptokinase** (I) [9002-01-1] gene (**skc**) of Streptococcus **equisimilis** H46A was **cloned** in Escherichia coli using vector λL47. One of the **recombinant clones** was used to subclone **skc** in E. coli plasmid vectors. Plasmids pMF2 (10.4 kilobases, composed of pACYC184 plus a 6.4-kilobase EcoRI fragment) and pMF5 (6.9 kilobases, with a 2.5-kilobase fragment in the PstI site of pBR322) determined I formation in E. coli; **expression** of **skc** was independent of its orientation, indicating that the complete gene, together with its control elements, was present. The 2.5-kilobase PstI fragment of pMF5 was isolated and sequenced in the M13 system. Of 2568 base pairs, the largest open reading frame consisted of 1320 base pairs coding for prestreptokinase, corresponding to I plus its 26-amino acid leader sequence. **Expression** of **skc** was attained in S. sanguis after transformation with the shuttle vector pSM752. In fermentation expts., I production rates of 1500 U/mL were attained, which was below the levels obtained with S. **equisimilis**. Use of pSM752 for similar transformation of Bacillus subtilis is briefly discussed.

L11 ANSWER 90 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 34
ACCESSION NUMBER: 1987:277792 BIOSIS
DOCUMENT NUMBER: PREV198784018831; BA84:18831
TITLE: MOLECULAR **CLONING** OF **STREPTOKINASE** GENE FROM STREPTOCOCCUS-**EQUISIMILIS** AND ITS **EXPRESSION** IN ESCHERICHIA-COLI.
AUTHOR(S): ROH D C [Reprint author]; KIM J H; PARK S K; LEE J W; BYRUN S M
CORPORATE SOURCE: DEP BIOLOGICAL SCIENCE AND ENGINEERING, KOREA ADVANCED INST SCIENCE AND TECHNOLOGY KAIST , PO BOX 150 CHONGRYANG, SEOUL 131, KOREA
SOURCE: Korean Biochemical Journal, (1986) Vol. 19, No. 4, pp. 391-398.
CODEN: KBCJAK. ISSN: 0368-4881.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 19 Jun 1987
Last Updated on STN: 19 Jun 1987
AB The streptococcal genomic DNA digested with Pst I was **cloned** in E. coli HB101. The overlay technique of casein/plasminogen was used to screen the **clones** for **recombinants** carrying the **streptokinase** gene. The insert size of the plasmid carrying the **streptokinase** gene was a 2.5, 4.3, and 5.8 Kb, respectively. The restriction maps of all three hybrid plasmids were constructed by

digestion with Pst I, Pvu II, Sal I, Hind III, Ava I, BamH I, and Cla I. For the identification of **cloned** gene, **streptokinase** was highly purified from S. **equisimilis** by the methods of gel chromatography and isoelectric focusing and rabbits were immunized with this purified **streptokinase**. Several lines of evidence, including proof obtained by the immunodiffusion technique, established that the enzyme from E. coli was identical to that from S. **equisimilis**. In the E. coli cell culture, we found the activity of **streptokinase** in all three principal locations of the cell. More than 50% were existed in the intracellular space.

L11 ANSWER 91 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1986:509637 HCAPLUS
DOCUMENT NUMBER: 105:109637
TITLE: The **streptokinase** gene
AUTHOR(S): Malke, H.; Ferretti, J. J.
CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Ther., Ger. Acad. Sci., Jena, DDR-6900, Ger. Dem. Rep.
SOURCE: Folia Haematologica (Leipzig) (1986), 113(1-2), 88-98
CODEN: FOHEAW; ISSN: 0323-4347
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Streptococcus **equisimilis** Gene **skc** for **streptokinase** [9002-01-1] was **cloned** on the vector L47 and then subcloned into plasmids pACYC184 and pBR322 to form **recombinant** plasmids pMF2 (10.4 kb) and pMF5 (9.9 kb), resp., for **expression** in Escherichia coli. Plasmid pMF5 contained a 2568-base-pair (bp) insert that included the 1320-bp coding sequence for prestreptokinase. The prestreptokinase comprised 440 amino acid residues, including a 26-amino acid signal peptide. The insert also contained the **skc** upstream regions involved in the regulation of transcription and translation and a 15-bp repeat located 34 bp downstream of the **skc** translation stop signal, which very likely represents the rho-independent transcription terminator. The **skc** gene showed no extended regions homologous to the staphylokinase gene. Heterologous **skc** gene **expression** was also attained in S. sanguis after subcloning of the gene of pMF5 onto plasmid pSM7 to form the bifunctional shuttle plasmid pSM752 (13.3 kb). Plasmid pSM752 was not only functional in E. coli and S. sanguis, but also in Bacillus subtilis. The **cloned streptokinase expressed** in E. coli, S. sanguis, or B. subtilis has the same specificity as that of the donor strain.

L11 ANSWER 92 OF 106 MEDLINE on STN DUPLICATE 35
ACCESSION NUMBER: 86281080 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3090231
TITLE: **Expression** of the **streptokinase** gene from Streptococcus **equisimilis** in Bacillus subtilis.
AUTHOR: Klessen C; Malke H
SOURCE: Journal of basic microbiology, (1986) 26 (2) 75-81.
Journal code: 8503885. ISSN: 0233-111X.
PUB. COUNTRY: GERMANY, EAST: German Democratic Republic
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198609
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19900321
Entered Medline: 19860916
AB The previously **cloned** and sequenced **streptokinase** gene (**skc**) from Streptococcus **equisimilis** H46A was inserted into plasmid vectors capable of replication in Bacillus subtilis. The **skc** gene was **expressed** by use of its own transcription

and translation signals which appeared to meet the stringent requirements of B. subtilis for efficient foreign gene **expression**. The secreted **streptokinase** activity began to decline toward the end of the exponential growth phase suggesting that B. subtilis exoproteases hydrolyzed and inactivated the foreign protein.

L11 ANSWER 93 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1985-11044 BIOTECHDS
TITLE: New plasmid transformed strain of Streptococcus sanguis; pduction of a new form of **streptokinase** for the treatment of thromboembolic disease
PATENT ASSIGNEE: Arzneim.Dresden
PATENT INFO: EP 150424 7 Aug 1985
APPLICATION INFO: EP 1984-115655 17 Dec 1984
PRIORITY INFO: DD 1983-258031 16 Dec 1983
DOCUMENT TYPE: Patent
LANGUAGE: German
OTHER SOURCE: WPI: 1985-191492 [32]
AB A new **streptokinase** producing strain of Streptococcus sanguis (Challis)(pSM 752) is claimed. This has been transformed with a **streptokinase** gene inserted from the donor strain of Streptococcus **equisimilis** H46A. Also new is a uniform monogenetic **streptokinase** of mol.weight 42,000 and isoelectric point of 5-5.5, formed by the cultivation of the new strain or other Streptococcus gp. H strains transformed with plasmid pSM 752. The new strain is cultivated in a liquid nutrient medium at 25-40 deg, preferably 30 deg at pH 6-8, preferably 7.2 for 10-36 hr, preferably 24 hr. The sugar content of the medium is maintained at 0.2-2, preferably 1% and the nitrogen content is 0.1-0.8%, preferably 0.2-0.4%. The **streptokinase** is useful for the treatment of thromboembolic vascular disorders and, since it is formed without contamination by streptococcal toxins, extensive purification is not required. (11pp)

L11 ANSWER 94 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1985-08513 BIOTECHDS
TITLE: Production of **streptokinase**; by cultivation of Escherichia coli ATCC 39613 containing **recombinant** plasmid PMF1
PATENT ASSIGNEE: Phillips-Petrol.
PATENT INFO: AU 8433859 18 Apr 1985
APPLICATION INFO: AU 1984-33859 5 Oct 1984
PRIORITY INFO: US 1984-585417 2 Mar 1984; DD 1983-255523 10 Oct 1983
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1985-135032 [23]
AB A **recombinant** vector plasmid pMF1 for the transformation of a host to produce **streptokinase** is new. The vector contains a polydeoxyribonucleotide fragment insert which codes for the synthesis and secretion of **streptokinase**. The transformant microorganism is preferably Escherichia coli HB101 and the vector, plasmid pBR322. The fragment coding for **streptokinase** synthesis and secretion is derived from a microorganism of the genus Streptococcus, especially Streptococcus **equisimilis** strain H46A and may have restriction endonuclease cleavage sites at the terminii e.g. it has 7400 bp and the cleavage sites are for HindIII. The **recombinant** vector is obtained by digestion of a vector with a restriction endonuclease to give linear DNA. This DNA is ligated to the **streptokinase** fragment to give the **recombinant**. This fragment is obtained by digestion of Streptococcus equilimilis with the same restriction endonuclease, especially Pst I, as is used to digest the initial vector. **Streptokinase** can be produced and isolated for use as a thrombolytic agent to facilitate the in vivo lysis or dissolution of blood clots. (28pp)

L11 ANSWER 95 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1985:608113 HCAPLUS
DOCUMENT NUMBER: 103:208113
TITLE: **Streptokinase**-coding **recombinant** vectors
INVENTOR(S): Ferretti, Joseph J.; Malke, Horst
PATENT ASSIGNEE(S): Phillips Petroleum Co. , USA
SOURCE: Eur. Pat. Appl., 21 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 151337	A2	19850814	EP 1984-306851	19841008
EP 151337	A3	19861008		
R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE				
DD 249037	A1	19870826	DD 1983-255523	19831010
US 4764469	A	19880816	US 1984-585417	19840302
AU 8433859	A1	19850418	AU 1984-33859	19841005
AU 561372	B2	19870507		
ZA 8407873	A	19850529	ZA 1984-7873	19841008
AT 61816	E	19910415	AT 1984-306851	19841008
FI 8403963	A	19850411	FI 1984-3963	19841009
NO 8404039	A	19850411	NO 1984-4039	19841009
DK 8404822	A	19850426	DK 1984-4822	19841009
JP 60237995	A2	19851126	JP 1984-212403	19841009
ES 536623	A1	19870116	ES 1984-536623	19841009
CA 1223223	A1	19870623	CA 1984-464939	19841009
DD 273284	A5	19891108	DD 1984-268254	19841010
US 5066589	A	19911119	US 1988-212254	19880627
US 5187098	A	19930216	US 1992-888420	19920522

PRIORITY APPLN. INFO.:

DD 1983-255523	A	19831010
US 1984-585417	A	19840302
EP 1984-306851	A	19841008
US 1988-212254	A2	19880627
US 1989-348206	B1	19890509

AB **Recombinant** vectors that code for **streptokinase** [9002-01-1] are constructed and **cloned** in Escherichia coli. Thus, DNA from Streptococcus **equisimilis** was isolated and digested with the restriction endonuclease Sau3A. DNA fragments of between 4-15 kb were **cloned** into phage λ L47. The ligated phage was infectively added to E. coli lawns and **streptokinase**-producing **clones** were isolated. The DNA from one such **clone**, λ L47E **skc** was partially digested with HindIII and then inserted into the HindIII site of plasmid pBR322. The **recombinant** plasmids were used to transform E. coli strain HB101. The plasmid isolated from 1 **streptokinase** -producing strain, pMF1, was isolated and a restriction map was prepared A nucleotide sequence anal. of pMF1 showed that the **cloned** fragment encoded for **streptokinase** as well as an amino-terminal signal peptide which is bound to **streptokinase** and which is hydrolyzed during a **streptokinase** secretion event.

L11 ANSWER 96 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1987:472094 HCAPLUS
DOCUMENT NUMBER: 107:72094
TITLE: New **cloning** vectors for Escherichia coli and Bacillus subtilis
INVENTOR(S): Klessen, Christian; Malke, Horst
PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Ger. Dem. Rep.
SOURCE: Ger. (East), 7 pp.

CODEN: GEXXA8
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 231083	A1	19851218	DD 1984-264255	19840619
PRIORITY APPLN. INFO.:			DD 1984-264255	19840619

AB New **cloning** vectors for Escherichia coli and Bacillus subtilis, derived from the bifunctional plasmid pGR71 by insertion of a promoter-containing DNA fragment into the unique HindIII site of pGR71 upstream of the chloramphenicol acetyltransferase gene, are described. The new plasmids pSM1711, pSM7711, and pSM7712 contain a Streptococcus promoter from plasmids pMF1, pSM10, or pSM7.

L11 ANSWER 97 OF 106 MEDLINE on STN DUPLICATE 36
ACCESSION NUMBER: 85232082 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2989113
TITLE: Nucleotide sequence of the **streptokinase** gene from Streptococcus **equisimilis** H46A.
AUTHOR: Malke H; Roe B; Ferretti J J
CONTRACT NUMBER: AI 9304 (NIAID)
SOURCE: Gene, (1985) 34 (2-3) 357-62.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-K02986
ENTRY MONTH: 198508
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 20000303
Entered Medline: 19850821

AB The entire nucleotide sequence of a **cloned** 2568-bp PstI fragment from the genome of Streptococcus **equisimilis** H46A encoding the **streptokinase** gene (**skc**) has been determined. The longest open reading frame comprises 1320 bp which code for **streptokinase**. The protein is synthesized with a 26-amino acid residue N-terminal extension having properties characteristic of a signal peptide. Comparison of the deduced amino acid sequence with the available amino acid sequence of a commercial **streptokinase** reveals minor primary structure differences. The nucleotide sequencing of **skc** does not support the hypothesis that the gene has evolved by duplication and fusion, as suggested by internal twofold amino acid homologies of its product. Furthermore, the **skc** gene sequence shows no extended regions homologous to the staphylokinase gene. Upstream from the **skc** gene, the putative **skc** promoter and the ribosome-binding site sequence have been identified; downstream from the coding region, inverted repeat sequences thought to function as transcription terminators have been detected.

L11 ANSWER 98 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1986:473464 HCAPLUS
DOCUMENT NUMBER: 105:73464
TITLE: Hybridization of a **cloned** group C streptococcal **streptokinase** gene with DNA from other streptococcal species
AUTHOR(S): Huang, T. T.; Malke, H.; Ferretti, J. J.
CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, USA
SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis.,

9th (1985), Meeting Date 1984, 234-6. Editor(s): Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi. Reedbooks: Bracknell, UK.
CODEN: 55BSAN

DOCUMENT TYPE: Conference
LANGUAGE: English

AB The previously **cloned streptokinase** [9002-01-1] gene (**skc**) of Streptococcus **equisimilis** and 2 subfragments were used as DNA hybridization probes to determine sequence homologies with other streptococcal species. The human pathogenic streptococci of strains A, C, and G were the only strains that had a pos. correlation between the ability to produce **streptokinase** and to hybridize with the gene **skc** DNA probe. In conjunction with other streptococcal DNA probes, such as streptolysin O, hyaluronidase, DNase, and erythrogenic toxins, the **skc** probe may be of diagnostic significance in the rapid identification of human pathogenic streptococci.

L11 ANSWER 99 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1986:473709 HCAPLUS
DOCUMENT NUMBER: 105:73709
TITLE: **Cloning** of streptococcal genes with Streptococcus-Escherichia coli shuttle vector pSA3
AUTHOR(S): Dao, M. L.; Ferretti, J. J.
CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, USA
SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis., 9th (1985), Meeting Date 1984, 233-4. Editor(s): Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi. Reedbooks: Bracknell, UK.
CODEN: 55BSAN

DOCUMENT TYPE: Conference
LANGUAGE: English

AB A shuttle vector, the chimeric plasmid pSA3, which can replicate in both E. coli and S. sanguis, was constructed. Chromosomal DNA from S. mutans was ligated into this plasmid and **cloned** in E. coli. Of 472 **clones** tested, 43 **clones expressed** S. mutans surface antigens. A **cloned** S. **equisimilis streptokinase** [9002-01-1] gene was inserted into plasmid pSA3 and then used to transform E. coli, S. sanguis, and S. mutans, all of which **expressed** the **cloned streptokinase** gene.

L11 ANSWER 100 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1986:473707 HCAPLUS
DOCUMENT NUMBER: 105:73707
TITLE: **Cloned streptokinase** gene from Streptococcus **equisimilis** H46A
AUTHOR(S): Malke, H.; Ferretti, J. J.
CORPORATE SOURCE: Ger. Acad. Sci., Jena, Ger. Dem. Rep.
SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis., 9th (1985), Meeting Date 1984, 221-2. Editor(s): Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi. Reedbooks: Bracknell, UK.
CODEN: 55BSAN

DOCUMENT TYPE: Conference
LANGUAGE: English

AB The **streptokinase** [9002-01-1] gene **skc** of S. **equisimilis** was **cloned** in Escherichia coli with plasmid pBR322. **Expression** of gene **skc** was observed with both orientations of the gene, which indicated that its own promoter was present and was functional in E. coli. **Streptokinase** was excreted by the E. coli host. The gene contained a 1320-base-pair open reading frame which encodes 440 amino acids, including a signal peptide of

26 amino acids.

L11 ANSWER 101 OF 106 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1985:107414 HCAPLUS
DOCUMENT NUMBER: 102:107414
TITLE: Streptococcus-Escherichia coli shuttle vector pSA3 and its use in the **cloning** of streptococcal genes
AUTHOR(S): Dao, My Lien; Ferretti, Joseph J.
CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, 73190, USA
SOURCE: Applied and Environmental Microbiology (1985), 49(1), 115-19
CODEN: AEMIDF; ISSN: 0099-2240
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A shuttle vector that can replicate in both Streptococcus and E. coli was constructed by joining the E. coli plasmid pACYC184 (chloramphenicol [56-75-7] and tetracycline [60-54-8] resistance) to the streptococcal plasmid pGB305 (erythromycin [114-07-8] resistance). The resulting chimeric plasmid is designated pSA3 (chloramphenicol, erythromycin, and tetracycline resistance) and had 7 unique restriction sites: EcoRI, EcoRV, BamHI, SalI, XbaI, NruI, and SphI. Mol. **cloning** into the EcoRI or EcoRV site results in inactivation of chloramphenicol resistance, and **cloning** into the BamHI, SalI site results in inactivation of tetracycline resistance in E. coli. Plasmid pSA3 was transformed and was stable in S. sanguis and S. mutans in the presence of erythromycin. Plasmid pSA3 was used to construct a library of the S. mutans GS5 genome in E. coli, and **expression** of surface antigens in this heterologous host was confirmed with S. mutans antiserum. A previously **cloned** determinant that species **streptokinase** [9002-01-1] was subcloned into pSA3, and this **recombinant** plasmid was stable in the presence of a selective pressure and **expressed streptokinase** activity in E. coli, S. sanguis, and S. mutans.

L11 ANSWER 102 OF 106 MEDLINE on STN DUPLICATE 37
ACCESSION NUMBER: 84221999 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6374659
TITLE: **Streptokinase: cloning, expression**, and excretion by Escherichia coli.
AUTHOR: Malke H; Ferretti J J
CONTRACT NUMBER: AI 9304 (NIAID)
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1984 Jun) 81 (11) 3557-61. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198407
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19970203
Entered Medline: 19840724
AB Genomic DNA from Streptococcus **equisimilis** strain H46A was **cloned** in Escherichia coli by using the bacteriophage lambda replacement vector L47 and an in vitro packaging system. A casein/plasminogen overlay technique was used to screen the phage bank for **recombinants** carrying the **streptokinase** gene (**skc**). The gene was present with a frequency of 1 in 836 **recombinants**, and 10 independent **clones** containing **skc** were isolated and physically characterized. One **recombinant clone** was used to subclone **skc** in E. coli plasmid vectors. Plasmid pMF2 [10.4 kilobases (kb)] consisting of

pACYC184 with a 6.4-kb H46A DNA fragment in the EcoRI site and pMF5 (6.9 kb) carrying a 2.5-kb fragment in the Pst I site of pBR322 were among the **recombinant** plasmids determining **streptokinase** production in three different E. coli host strains. **Expression** of **skc** was independent of its orientation in either vector, indicating that its own promoter was present and functional in E. coli. However, **expression** in pBR322 was more efficient in one orientation than in the other, suggesting that one or both of the bla gene promoters contributed to **skc expression**. Several lines of evidence, including proof obtained by the immunodiffusion technique, established the identity of E. coli **streptokinase**. Testing cell-free culture supernatant fluids, osmotic shock fluids, and sonicates of osmotically shocked cells for **streptokinase** activity revealed the substance to be present in all three principal locations, indicating that E. coli cells were capable of releasing substantial amounts of **streptokinase** into the culture medium.

L11 ANSWER 103 OF 106 MEDLINE on STN DUPLICATE 38
ACCESSION NUMBER: 85035880 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6593564
TITLE: **Expression** of a **streptokinase** gene from Streptococcus **equisimilis** in Streptococcus sanguis.
AUTHOR: Malke H; Gerlach D; Kohler W; Ferretti J J
SOURCE: Molecular & general genetics : MGG, (1984) 196 (2) 360-3. Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198412
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19900320
Entered Medline: 19841218

AB Using **recombinant** DNA techniques, we introduced a previously **cloned streptokinase** gene from Streptococcus **equisimilis** into the Challis strain of S. sanguis (group H). The gene was **expressed** in the new host under the control of its own promoter and the gene product had biological properties identical to authentic **streptokinase**. However, the molecular weight of **cloned streptokinase** (42 K) as **expressed** by S. sanguis was substantially lower than that of authentic **streptokinase** (47 K). Since the **cloned streptokinase** gene encoded a 47 K mature protein, the lowered molecular weight of S. sanguis **streptokinase** may reflect posttranslational proteolytic cleavage, which leaves the biological activity of the gene product and its serological reactivity unimpaired.

L11 ANSWER 104 OF 106 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1984-03868 BIOTECHDS
TITLE: **Streptokinase: cloning expression** and excretion by E.coli; using Streptococcus **equisimilis** genomic DNA (conference abstract)
AUTHOR: Malke H; Ferretti J J
LOCATION: Acad. Sci. GDR, Jena, DDR.
SOURCE: Abstr.Annu.Meet.Am.Soc.Microbiol; (1984) 84 Meet., 67
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Genomic DNA from Streptococcus **equisimilis** H46A (group C) was **cloned** into Escherichia coli using the lambda replacement vector L47 and an in vitro packaging system. The phage bank was screened for **recombinants** containing the **streptokinase** (**skc**) gene by the casein-plasminogen overlay technique. 10 Independent

clones containing the **skc** gene were isolated and one was used to subclone the **skc** gene into E.coli plasmid vectors pBR322 and pACYC184. Plasmid pMF2 and pMF5 were among the **recombinant** plasmids determining **streptokinase** production in 3 different E.coli host strains. **Expression** of **skc** was independent of its orientation in either vector, indicating that its own promoter was present and functional in E.coli. Analysis of cell free culture supernatant fluids, osmotic shockates, and sonicates of osmotically shocked cells for **streptokinase** activity revealed the substance to be present in all 3 locations, indicating that E.coli cells were capable of releasing substantial amounts of **streptokinase** into the culture medium. (0 ref)

L11 ANSWER 105 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1984:100214 BIOSIS
DOCUMENT NUMBER: PREV198427016706; BR27:16706
TITLE: HYBRIDIZATION OF A **CLONED** GROUP C STREPTOCOCCAL STREPTO KINASE GENE WITH DNA FROM OTHER STREPTOCOCCAL SPECIES.
AUTHOR(S): HUANG T-T [Reprint author]; WEEKS C R; MALKE H; FERRETTI J J
CORPORATE SOURCE: UNIV OKLA HEALTH SCI CENT, OKLAHOMA CITY, OKLA, USA
SOURCE: Abstracts of the Annual Meeting of the American Society for Microbiology, (1984) Vol. 84, pp. ABSTRACT D75.
Meeting Info.: 84TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, ST. LOUIS, MO., USA, MAR. 4-9, 1984.
ABSTR ANNU MEET AM SOC MICROBIOL.
CODEN: ASMACK. ISSN: 0094-8519.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH

L11 ANSWER 106 OF 106 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1984:92832 BIOSIS
DOCUMENT NUMBER: PREV198427009324; BR27:9324
TITLE: STREPTO KINASE **CLONING EXPRESSION** AND EXCRETION BY ESCHERICHIA-COLI.
AUTHOR(S): MALKE H [Reprint author]; FERRETTI J J
CORPORATE SOURCE: ACAD SCI GDR, JENA, GDR
SOURCE: Abstracts of the Annual Meeting of the American Society for Microbiology, (1984) Vol. 84, pp. ABSTRACT D97.
Meeting Info.: 84TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, ST. LOUIS, MO., USA, MAR. 4-9, 1984.
ABSTR ANNU MEET AM SOC MICROBIOL.
CODEN: ASMACK. ISSN: 0094-8519.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH

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(FILE 'HOME' ENTERED AT 17:11:46 ON 29 DEC 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005

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L1        42118 S STREPTOKINASE?
L2          630 S "SKC" OR "SKC-2"
L3        42539 S L1 OR L2
L4      7454183 S CLON? OR EXPRESS? OR RECOMBINANT
L5         4154 S L3 AND L4
L6         1362 S EQUISIMILIS
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L7          220 S L5 AND L6
L8        49433 S INCLUSION (W) BOD?
L9            5 S L7 AND L8
L10           1 DUP REM L9 (4 DUPLICATES REMOVED)
L11         106 DUP REM L7 (114 DUPLICATES REMOVED)
L12           5 S LAMNDA

=> s l11 and inclusion
L13           1 L11 AND INCLUSION

=> s l11 and aggregat?
L14           0 L11 AND AGGREGAT?

=> s l11 and insoluble
L15           0 L11 AND INSOLUBLE

=> s l11 and solubl?
L16           1 L11 AND SOLUBL?

=> s l11 and coli
L17          71 L11 AND COLI

=> d 1-71 ibib ab

L17  ANSWER 1 OF 71     MEDLINE on STN
ACCESSION NUMBER:   2002322701     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 12065504
TITLE:              Dual control of streptokinase and streptolysin S
                    production by the covRS and fasCAX two-component regulators
                    in Streptococcus dysgalactiae subsp. equisimilis.
AUTHOR:             Steiner Kerstin; Malke Horst
CORPORATE SOURCE:   Institute for Molecular Biology, Friedrich Schiller
                    University Jena, D-07745 Jena, Germany.
SOURCE:             Infection and immunity, (2002 Jul) 70 (7) 3627-36.
                    Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY:       United States
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
OTHER SOURCE:       GENBANK-AY075106; GENBANK-AY075107
ENTRY MONTH:        200207
ENTRY DATE:         Entered STN: 20020615
                    Last Updated on STN: 20020731
                    Entered Medline: 20020730
AB   Synthesis of the plasminogen activator streptokinase (SK) by
     group A streptococci (GAS) has recently been shown to be subject to
     control by two two-component regulators, covRS (or csrRS) and fasBCA.  In
     independent studies, response regulator CovR proved to act as the
     repressor, whereas FasA was found to act indirectly as the activator by
     controlling the expression of a stimulatory RNA, fasX.  In an
     attempt at understanding the regulation of SK production in the human
     group C streptococcal (GCS) strain H46A, the strongest SK producer known
     yet, we provide here physical and functional evidence for the presence of
     the cov and fas systems in GCS as well and, using a mutational approach,
     compare the balance between their opposing actions in H46A and GAS strain
     NZ131.  Sequence analysis combined with Southern hybridization revealed
     that the covRS and fasCAX operons are preserved at high levels of primary
     structure identity between the corresponding GAS and GCS genes, with the
     exception of fasB, encoding a second sensor kinase that is not a member of
     the GCS fas operon.  This analysis also showed that wild-type H46A is
     actually a derepressed mutant for SK and streptolysin S (SLS) synthesis,
     carrying a K102 amber mutation in covR.  Using cov and fas mutations in
     various combinations together with strain constructs allowing
     complementation in trans, we found that, in H46A, cov and fas contribute
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to approximately equal negative and positive extents, respectively, to constitutive SK and SLS activity. The amounts of SK paralleled the level of **skc**(H46A) transcription. The most profound difference between H46A and NZ131 regarding the relative activities of the cov and fas systems consisted in significantly higher activity of a functional CovR repressor in NZ131 than in H46A. In NZ131, CovR decreased SK activity in a Fas(+) background about sevenfold, compared to a 1.9-fold reduction of SK activity in H46A. Combined with the very short-lived nature of covR mRNA (decay rate, 1.39/min), such differences may contribute to strain-specific peculiarities of the **expression** of two prominent streptococcal virulence factors in response to environmental changes.

L17 ANSWER 2 OF 71 MEDLINE on STN
ACCESSION NUMBER: 2002053807 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11779212
TITLE: Specificity role of the **streptokinase** C-terminal domain in plasminogen activation.
AUTHOR: Kim Dong Min; Lee Sang Jun; Yoon Suk Kwon; Byun Si Myung
CORPORATE SOURCE: Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), 305-701 Taejon, South Korea.
SOURCE: Biochemical and biophysical research communications, (2002 Jan 11) 290 (1) 585-8.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20020125
Last Updated on STN: 20020226
Entered Medline: 20020225

AB Several pathogenic bacteria secrete plasminogen activator proteins. **Streptokinase** (SKe) produced by Streptococcus **equisimilis** and staphylokinase secreted from Staphylococcus aureus are human plasminogen activators and **streptokinase** (SKu), produced by Streptococcus uberis, is a bovine plasminogen activator. Thus, the fusion proteins among these activators can explain the function of each domain of SKe. Replacement of the SKalpha domain with staphylokinase donated the staphylokinase-like activation activity to SKe, and the SKbetagamma domain played a role of nonproteolytic activation of plasminogen. **Recombinant** SKu also activated human plasminogen by staphylokinase-like activation mode. Because SKu has homology with SKe, the bovine plasminogen activation activities of SKe fragments were checked. SKebetagamma among them had activation activity with bovine plasminogen. This means that the C-terminal domain (gamma-domain) of **streptokinase** determines plasminogen species necessary for activation and converses the ability of substrate recognition to human species.

L17 ANSWER 3 OF 71 MEDLINE on STN
ACCESSION NUMBER: 2000038313 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10569766
TITLE: **Cloning**, **expression**, sequence analysis, and characterization of **streptokinases** secreted by porcine and equine isolates of Streptococcus **equisimilis**.
AUTHOR: Caballero A R; Lottenberg R; Johnston K H
CORPORATE SOURCE: Department of Microbiology, Immunology and Parasitology, Louisiana State University Medical Center, New Orleans, Louisiana 70112, USA.
CONTRACT NUMBER: R01DK45014 (NIDDK)

SOURCE: Infection and immunity, (1999 Dec) 67 (12) 6478-86.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF104300; GENBANK-AF104301
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991220

AB **Streptokinases** secreted by nonhuman isolates of group C streptococci (Streptococcus equi, S. **equisimilis**, and S. zooepidemicus) have been shown to bind to different mammalian plasminogens but exhibit preferential plasminogen activity. The **streptokinase** genes from S. **equisimilis** strains which activated either equine or porcine plasminogen were **cloned**, sequenced, and **expressed** in Escherichia **coli**. The **streptokinase** secreted by the equine isolate had little similarity to any known **streptokinases** secreted by either human or porcine isolates. The **streptokinase** secreted by the porcine isolate had limited structural and functional similarities to **streptokinases** secreted by human isolates. Plasminogen activation studies with immobilized (His)(6)-tagged **recombinant streptokinases** indicated that these **recombinant streptokinases** interacted with plasminogen in a manner similar to that observed when **streptokinase** and plasminogen interact in the fluid phase. Analysis of the cleavage products of the **streptokinase** -plasminogen interaction indicated that human, equine, and porcine plasminogens were all cleaved at the same highly conserved site. The site at which **streptokinase** was cleaved to form altered **streptokinase** (Sk*) was also determined. This study confirmed not only the presence of **streptokinases** in nonhuman S. **equisimilis** isolates but also that these proteins belong to a family of plasminogen activators more diverse than previously thought.

L17 ANSWER 4 OF 71 MEDLINE on STN
ACCESSION NUMBER: 1998350778 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9686161
TITLE: **Cloning**, **expression** and purification of **recombinant streptokinase**: partial characterization of the protein **expressed** in Escherichia **coli**.
AUTHOR: Avilan L; Yarzabal A; Jurgensen C; Bastidas M; Cruz J; Puig J
CORPORATE SOURCE: Laboratorio de Biologia y Medicina Experimental, Facultad de Ciencias, Universidad de Los Andes, Merida, Venezuela.
SOURCE: Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica ... [et al.], (1997 Dec) 30 (12) 1427-30.
Journal code: 8112917. ISSN: 0100-879X.
PUB. COUNTRY: Brazil
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19981020
Last Updated on STN: 19981020
Entered Medline: 19981005

AB We **cloned** the **streptokinase** (STK) gene of Streptococcus **equisimilis** in an **expression** vector of Escherichia **coli** to overexpress the profibrinolytic protein under the control of a tac promoter. Almost all the **recombinant**

STK was exported to the periplasmic space and recovered after gentle lysozyme digestion of induced cells. The periplasmic fraction was chromatographed on DEAE Sepharose followed by chromatography on phenyl-agarose. Active proteins eluted between 4.5 and 0% ammonium sulfate, when a linear gradient was applied. Three major STK derivatives of 47.5 kDa, 45 kDa and 32 kDa were detected by Western blot analysis with a polyclonal antibody. The 32-kDa protein formed a complex with human plasminogen but did not exhibit Glu-plasminogen activator activity, as revealed by a zymographic assay, whereas the 45-kDa protein showed a K(m) = 0.70 microM and kcat = 0.82 s-1, when assayed with a chromogen-coupled substrate. These results suggest that these proteins are putative fragments of STK, possibly derived from partial degradation during the export pathway or the purification steps. The 47.5-kDa band corresponded to the native STK, as revealed by peptide sequencing.

L17 ANSWER 5 OF 71 MEDLINE on STN
ACCESSION NUMBER: 96397500 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8804394
TITLE: **Cloning** of heterologous genes specifying detrimental proteins on pUC-derived plasmids in Escherichia **coli**.
AUTHOR: Muller J; van Dijl J M; Venema G; Bron S
CORPORATE SOURCE: Institut fur Molekularbiologie, Friedrich-Schiller-Universitat Jena, Germany.
SOURCE: Molecular & general genetics : MGG, (1996 Aug 27) 252 (1-2) 207-11.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 19961219
Entered Medline: 19961031

AB A system is described that enables the **cloning** of genes specifying detrimental proteins in Escherichia **coli**. The system is based on pUC plasmids and was developed for the **expression** of the Bacillus subtilis csaA gene, which is lethal when **expressed** at high levels. Suppressor strains that tolerate the presence of plasmids for high-level **expression** of csaA were isolated, which contained small cryptic deletion variants of the parental plasmid in high copy numbers. The cryptic plasmids consisted mainly of the pUC replication functions and lacked the csaA region and selectable markers. The co-resident, incompatible, cryptic plasmids enabled the maintenance of the csaA plasmids by reducing their copy number 20-fold, which resulted in a concomitant 3- to 7-fold reduction in the **expression** of plasmid-encoded genes. Strains carrying these cryptic endogenous plasmids proved to be useful for the construction of pUC-based **recombinant** plasmids carrying other genes, such as the **skc** gene of Streptococcus **equisimilis**, which cannot be **cloned** in high copy numbers in E. **coli**. Several strategies to reduce production levels of heterologous proteins specified by plasmids are compared.

L17 ANSWER 6 OF 71 MEDLINE on STN
ACCESSION NUMBER: 96396845 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8803948
TITLE: Structural dissection and functional analysis of the complex promoter of the **streptokinase** gene from Streptococcus **equisimilis** H46A.
AUTHOR: Grafe S; Ellinger T; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Medical microbiology and immunology, (1996 May) 185 (1)

11-7.
Journal code: 0314524. ISSN: 0300-8584.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970131

AB The overlapping tandem promoters of the **streptokinase** gene, P1 and P2, identified previously by S1 nuclease transcript mapping were functionally dissected by mutagenesis of their -10 regions and fused transcriptionally with or without the 202-bp upstream region (USR) to the luciferase reporter gene (luc) from Photinus pyralis to analyze the contribution of the different sequence elements to promoter activity in Escherichia **coli** and the homologous Streptococcus **equisimilis** strain H46A. In E. **coli**, virtually the entire promoter activity derived from the upstream promoter P1. In S. **equisimilis**, luc **expression** increased in the following order of the involved sequence elements: P2 approximately equal to P2 + USR < P1 < P1 + P2 < P1 + USR < P1 + P2 + USR. This shows that (1) in the homologous system, P1 and P2 alone are extremely weak, (2) in the USR-less arrangement, only the combined core promoters have substantial activity, and (3) the USR stimulates only P1 and the combination of P1 + P2. Thus, the tandem promoters presumably function by mutual contributary action and their full activity strongly depends on the AT-rich and statically bent upstream region. The distinctive feature determining the strength of P1 in both hosts appears to be its extended -10 region which matches the consensus TRTGN established for strong S. pneumoniae and Bacillus subtilis promoters.

L17 ANSWER 7 OF 71 MEDLINE on STN
ACCESSION NUMBER: 96305364 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8706717
TITLE: **Cloning**, sequencing and functional overexpression of the Streptococcus **equisimilis** H46A gapC gene encoding a glyceraldehyde-3-phosphate dehydrogenase that also functions as a plasmin(ogen)-binding protein. Purification and biochemical characterization of the protein.
AUTHOR: Gase K; Gase A; Schirmer H; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: European journal of biochemistry / FEBS, (1996 Jul 1) 239 (1) 42-51.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X97788
ENTRY MONTH: 199609
ENTRY DATE: Entered STN: 19960919
Last Updated on STN: 19990129
Entered Medline: 19960910

AB We previously identified DNA sequences involved in the function of the complex promoter of the **streptokinase** gene from Streptococcus **equisimilis** H46A, a human serogroup C strain known to **express** this gene at a high level. As a prerequisite to understanding possible mechanisms that control the balance between the plasminogen activating and plasmin(ogen) binding capacities of H46A, we describe here its gapC gene encoding glyceraldehyde-3-phosphate dehydrogenase (GraP-DH, EC 1.2.1.12), a glycolytic enzyme apparently transported to the cell surface where it functions as a

plasmin(ogen).binding protein. The gapC gene was **cloned** and sequenced and found to code for a 336-amino-acid polypeptide (approximately 35.9 kDa) exhibiting 94.9% sequence identity to the Plr protein from Streptococcus pyogenes shown by others to be capable of plasmin binding [Lottenberg, R., Broder, C. C., Boyle, M. D., Kain, S. J., Schroeder, B. L. & Curtiss, R. III (1992) J. Bacteriol. 174, 5204-5210]. To study the properties of the GapC protein, its gene was inducibly overexpressed in Escherichia **coli** from QIAexpress **expression** plasmids to yield the authentic GapC or (His)6GapC carrying a hexahistidyl N-terminus to permit affinity purification. Both proteins were functionally active, exhibiting specific GraP-DH activities of about 80 kat/mol (approximately 130 U/mg) after purification. Their binding parameters [association (ka) and dissociation (kd) rate constants, and equlibrium dissociation constants (Kd = kd/ka)] for the interaction with human Gluplasminogen and plasmin were determined by real-time biospecific interaction analysis using the Pharmacia BIAcore instrument. For comparative purposes, the commercial GraP-DH from Bacillus stearothermophilus (BstGraP-DH), a nonpathogenic organism, was included in these experiments. The Kd values for binding of plasminogen to GapC, (His)6GapC and BstGraP-DH were 220 nM, 260 nM and 520 nM, respectively, as compared to 25 nM, 17 nM and 98 nM, respectively, for the binding to plasmin. These data show that both the zymogen and active enzyme possess low-affinity binding sites for the gapC gene product and that the hexahistidyl terminus does not affect its function. Prior limited treatment with plasmin enhanced the subsequent plasminogen binding capacity of all three GraP-DHs, presumably by the exposure of new C-terminal lysine residues for binding to the zymogen.

L17 ANSWER 8 OF 71 MEDLINE on STN
ACCESSION NUMBER: 96200111 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8631718
TITLE: Functional analysis of a relA/spoT gene homolog from Streptococcus **equisimilis**.
AUTHOR: Mechold U; Cashel M; Steiner K; Gentry D; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Journal of bacteriology, (1996 Mar) 178 (5) 1401-11.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960715
Last Updated on STN: 19970203
Entered Medline: 19960703

AB We examined the functional attributes of a gene encountered by sequencing the **streptokinase** gene region of Streptococcus **equisimilis** H46A. This gene, originally called rel, here termed relS. **equisimilis**, is homologous to two related Escherichia **coli** genes, spoT and relA, that function in the metabolism of guanosine 5',3'-polyphosphates [(p)ppGpp]. Studies with a variety of E. **coli** mutants led us to deduce that the highly **expressed** rel S. **equisimilis** gene encodes a strong (p)ppGppase and a weaker (p)ppGpp synthetic activity, much like the spoT gene, with a net effect favoring degradation and no complementation of the absence of the relA gene. We verified that the Rel S. **equisimilis** protein, purified from an E. **coli** relA spoT double mutant, catalyzed a manganese-activated (p)ppGpp 3'-pyrophosphohydrolase reaction similar to that of the SpoT enzyme. This Rel S. **equisimilis** protein preparation also weakly catalyzed a ribosome-independent synthesis of (p)ppGpp by an ATP to GTP 3'-pyrophosphoryltransferase reaction when degradation was restricted by the absence of manganese ions. An analogous activity has been deduced for the SpoT protein from genetic evidence. In addition, the Rel S. **equisimilis** protein displays immunological

cross-reactivity with polyclonal antibodies specific for SpoT but not for RelA. Despite assignment of rel S. **equisimilis** gene function in E. **coli** as being similar to that of the native spoT gene, disruptions of rel S. **equisimilis** in S. **equisimilis** abolish the parental (p)ppGpp accumulation response to amino acid starvation in a manner expected for relA mutants rather than spoT mutants.

L17 ANSWER 9 OF 71 MEDLINE on STN
ACCESSION NUMBER: 96001243 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7557478
TITLE: Secretion of **streptokinase** fusion proteins from Escherichia **coli** cells through the hemolysin transporter.
AUTHOR: Kern I; Ceglowski P
CORPORATE SOURCE: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa.
SOURCE: Gene, (1995 Sep 22) 163 (1) 53-7.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199511
ENTRY DATE: Entered STN: 19951227
Last Updated on STN: 20021008
Entered Medline: 19951108

AB The hemolysin (HlyA) secretion system was used to achieve the sec-independent secretion of **streptokinase** (**Skc**) originating from Streptococcus **equisimilis** into the medium by Escherichia **coli** cells. The in-frame fusions of the **skc** gene, either possessing or lacking a region encoding the signal peptide (SP) with the 3'-end of the hlyA gene of various lengths were analysed. All hybrids retained **Skc** activity. Hybrid proteins devoided of the N-terminal SP, regardless of length of the hlyA secretion signal (62 vs. 194 amino acids), were secreted into the medium by the E. **coli** HlyA transporter at similar levels. Considerable amounts of hybrid proteins were still, however, associated with E. **coli** cells, mainly in the degraded form.

L17 ANSWER 10 OF 71 MEDLINE on STN
ACCESSION NUMBER: 95342169 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7616967
TITLE: Complex transcriptional control of the **streptokinase** gene of Streptococcus **equisimilis** H46A.
AUTHOR: Gase K; Ellinger T; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Molecular & general genetics : MGG, (1995 Jun 25) 247 (6) 749-58.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950905
Last Updated on STN: 19950905
Entered Medline: 19950822

AB On the Streptococcus **equisimilis** H46A chromosome, the divergent coding sequences of the genes for the plasminogen activator **streptokinase** (**skc**) and a leucine-rich protein (lrp), the function of which is unknown, are separated by a 328 bp intrinsically bent DNA region rich in AT tracts. To begin to understand the **expression** control of these two genes, we mapped their

transcriptional initiation sites by S1 nuclease analysis and studied the influence of the bent intergenic region on promoter strength, using promoter-reporter gene fusions of **skc**' and lrp' to 'lacZ from Escherichia **coli**. The major transcriptional start sites, in both S. **equisimilis** and E. **coli**, mapped 22 bases upstream of the ATG start site of lrp (G), and 24 and 32 bases upstream of the translational initiation codon of **skc** (A and G, respectively), indicating the existence of two overlapping canonical **skc** promoters arranged in tandem on opposite faces of the helix. The reporter gene fusions were **cloned** in E. **coli** on a vector containing a 1.1 kb fragment of the S. **equisimilis** dexB gene, thus allowing promoter strength to be measured in multiple plasmid-form copies in the heterologous host and in single-copy genomic form following integration into the **skc** region of the homologous host. In S. **equisimilis**, **skc**'-'lacZ was **expressed** about 200-fold more strongly than the corresponding lrp'-'lacZ fusion. In contrast, in E. **coli**, the corresponding levels of **expression** differed by only about 11-fold. Deletion of the 202 bp bent region upstream of the **skc** and lrp core promoters caused a 13-fold decrease in **skc** promoter activity in S. **equisimilis** but did not alter lrp promoter strength in this host. In contrast, when studied in E. **coli**, this deletion did not alter the strength of the **skc**-double promoter and even increased by 2.4- to 3-fold the activity of the lrp promoter. This comparative promoter analysis shows that **skc** has a complex promoter structure, the activity of which in the homologous genomic environment specifically depends on sequences upstream of the two core promoters. Thus, the **skc** promoter structure resembles that of an array of promoters involved in a transcriptional switch; however, the nature of the potential switch factor(s) remains unknown.

L17 ANSWER 11 OF 71 MEDLINE on STN
ACCESSION NUMBER: 95157528 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7531815
TITLE: Transcription termination of the **streptokinase** gene of Streptococcus **equisimilis** H46A: bidirectionality and efficiency in homologous and heterologous hosts.
AUTHOR: Steiner K; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Molecular & general genetics : MGG, (1995 Feb 6) 246 (3) 374-80.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199503
ENTRY DATE: Entered STN: 19950322
Last Updated on STN: 19960129
Entered Medline: 19950316

AB In Streptococcus **equisimilis** H46A, a hypersymmetrical transcription terminator with bidirectional activity was localized between the translational termination codons of the **streptokinase** gene, **skc**, and the rel-orf1 genes. These two transcription units are oriented towards each other, and under normal conditions the **skc** mRNA level exceeds that of the rel-orf1 genes by a factor of at least 1000. Reporter vectors based on the promoterless cat gene were constructed by transcriptional fusion of **skc** to cat, such that the region between the two genes contained the terminator in **skc** orientation or in rel-orf1 orientation. Additionally, **skc** and cat were fused directly, with deletion of the terminator. The reporter vectors were designed to be capable of being studied either as multicopy plasmids in Escherichia **coli** or in single copy following

integration, via **skc**, into the S. **equisimilis** chromosome. Chloramphenicol acetyl transferase (CAT) activity assays in conjunction with determination of chloramphenicol resistance levels and Northern hybridization analysis showed that the terminator is active in either host and orientation. However, termination efficiency was host dependent, with high terminator strength being observed in the homologous streptococcal background and appreciable readthrough occurring in E. **coli**. The extent of transcriptional readthrough was dependent upon terminator orientation, with termination being more efficient in rel-orf1 polarity. The results suggest that, in S. **equisimilis**, transcription of both **skc** and rel-orf1 is efficiently terminated by a common signal, and that these genes are largely protected from convergent transcription, which otherwise would seem to be particularly detrimental to the weakly **expressed** rel-orf1 genes.

L17 ANSWER 12 OF 71 MEDLINE on STN
ACCESSION NUMBER: 94049672 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8232196
TITLE: Genetic organization of the **streptokinase** region of the Streptococcus **equisimilis** H46A chromosome.
AUTHOR: Mechold U; Steiner K; Vettermann S; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Molecular & general genetics : MGG, (1993 Oct) 241 (1-2) 129-40.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X72832
ENTRY MONTH: 199312
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 19940117
Entered Medline: 19931215

AB The complete nucleotide sequences of four genes and one open reading frame (ORF1) adjacent to the **streptokinase** gene, **skc**, from Streptococcus **equisimilis** H46A were determined. These genes are encoded on the opposite DNA strand to **skc** and are arranged as follows: dexB-abc-lrp-**skc**-ORF1-rel. The dexB gene, coding for an alpha-glucosidase (M(r) 61,733), and abc, encoding an ABC transporter (M(r) 42,080), are similar to the dexB and msmK genes, respectively, from the multiple sugar metabolism operon of S. mutans. The lrp gene specifies a leucine-rich protein (M(r) 32,302) that has a leucine-zipper motif at its C-terminus. The function of the Lrp protein is not known but appeared to be detrimental when overexpressed in Escherichia **coli**. Although lrp appears not to be an essential gene, as judged by plasmid insertion mutagenesis, it is conserved in all streptococcal strains carrying a **streptokinase** gene. The rel gene showed significant homology to the E. **coli** relA and spoT genes involved in the stringent response to amino acid deprivation. Multiple alignment of the amino acid sequences of Rel (M(r) 83,913), RelA and SpoT revealed 59.4% homology of the primary structures. Northern hybridization analyses of the genes in the **skc** region showed **skc** to be transcribed most abundantly. In addition to transcripts for **skc** , monocistronic mRNAs were detected for all three genes divergently transcribed from **skc**. Although there was also some read-through transcription from lrp into abc, and from abc into dexB, the transcription pattern suggests a high degree of transcriptional and functional independence not only of **skc** but also abc and dexB. Prominent structural features in intergenic regions included a static DNA bending locus located upstream and a putative bidirectional transcription terminator downstream of **skc**.

L17 ANSWER 13 OF 71 MEDLINE on STN

ACCESSION NUMBER: 92039051 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1937032
TITLE: Isolation, sequence and **expression** in Escherichia **coli**, Bacillus subtilis and Lactococcus lactis of the DNase (streptodornase)-encoding gene from Streptococcus **equisimilis** H46A.
AUTHOR: Wolinowska R; Ceglowski P; Kok J; Venema G
CORPORATE SOURCE: Department of Pharmaceutical Microbiology, Medical Academy, Warsaw, Poland.
SOURCE: Gene, (1991 Sep 30) 106 (1) 115-9.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M59725; GENBANK-M59726; GENBANK-M59727; GENBANK-M59728; GENBANK-M63990; GENBANK-S61507; GENBANK-S63856; GENBANK-S63863; GENBANK-S65020; GENBANK-S65060; GENBANK-X17241
ENTRY MONTH: 199112
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19920124
Entered Medline: 19911223

AB A partial library of BclI-generated chromosomal DNA fragments from Streptococcus **equisimilis** H64A (Lancefield Group C) was constructed in Escherichia **coli**. **Clones** displaying either **streptokinase** or deoxyribonuclease (streptodornase; SDC) activities were isolated. The gene (sdc) **expressing** the SDC activity was allocated on the 1.1-kb AccI DNA subfragment. Sequence analysis of this DNA fragment revealed the presence of one open reading frame, which could encode a protein of 36.8 kDa. The N-terminal portion of the deduced protein exhibited features characteristic of prokaryotic signal peptides. The sdc gene was **expressed** in E. **coli** , Bacillus subtilis and Lactococcus lactis. As observed for S. **equisimilis**, in the heterologous Gram + hosts, at least part of the SDC protein was secreted into the medium.

L17 ANSWER 14 OF 71 MEDLINE on STN
ACCESSION NUMBER: 90172183 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2625666
TITLE: Sequence-directed DNA bending upstream of the **streptokinase** promoter.
AUTHOR: Muller J; Malke H
CORPORATE SOURCE: Akademie der Wissenschaften der DDR.
SOURCE: Journal of basic microbiology, (1989) 29 (9) 611-6.
Journal code: 8503885. ISSN: 0233-111X.
PUB. COUNTRY: GERMANY, EAST: German Democratic Republic
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199004
ENTRY DATE: Entered STN: 19900601
Last Updated on STN: 19900601
Entered Medline: 19900409

AB A 450-base pair (bp) HinfI restriction fragment from the chromosome of Streptococcus **equisimilis** H46A contains the early coding region of the **streptokinase** gene (**skc**), the **skc** promoter, and a stretch of DNA 5' to the--35 region of the **skc** promoter. Two-dimensional polyacrylamide (PA) gel electrophoresis at two different temperatures showed that this fragment migrates anomalously slowly on PA gels, suggesting the existence of a bent DNA conformation. Inspection of the nucleotide sequence confirmed this suggestion by revealing numerous oligomeric dA.dT tracts, some of which are in phase with the helix screw. Computer analysis of the sequence predicted the

existence of two bending loci, one of which is located upstream of the **skc** promoter. In addition to showing DNA bending, the 450-bp HinfI fragment contains multiple 13-bp sequences homologous to the Escherichia **coli** integration host factor DNA-binding consensus sequence. Insertion of IS1 into a site immediately upstream of the--35 region decreased the **expression** level of **skc** in E. **coli**, suggesting that DNA conformation upstream of the promoter has a role in **skc expression**.

L17 ANSWER 15 OF 71 MEDLINE on STN
ACCESSION NUMBER: 88302119 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3043172
TITLE: Tripartite **streptokinase** gene fusion vectors for gram-positive and gram-negative procaryotes.
AUTHOR: Klessen C; Schmidt K H; Ferretti J J; Malke H
CORPORATE SOURCE: Academy of Sciences of the GDR, Central Institute of Microbiology and Experimental Therapy, Jena.
SOURCE: Molecular & general genetics : MGG, (1988 May) 212 (2) 295-300.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198809
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19900308
Entered Medline: 19880921

AB A specific 1,596 bp HincII fragment ('**skc**) from the chromosome of Streptococcus **equisimilis** contains an active **streptokinase** (SK) gene (**skc**) lacking, in addition to the **expression** signals, codons 1 through 39 of wild-type **skc** but retaining the remainder of the **skc** coding sequence together with the transcription terminator. Using this fragment as an indicator gene, we constructed two types of vectors which in appropriate hosts resulted in the synthesis of SK fusion proteins after insertional activation of '**skc**. The first type are open reading frame (ORF) vectors in which '**skc** was inserted into pUC18 out of frame with respect to lacZ', thus conferring an SK-negative phenotype. Any DNA fragments representing ORFs inserted between the lacZ' **expression** signals and '**skc** such that the **skc** reading frame was restored resulted in the production of tripartite proteins which exhibited SK activity. The second type of vector, which functioned in both gram-positive and gram-negative bacteria, used the streptococcal speA **expression** and secretion signals in front of the ORF to activate '**skc** insertionally. Using a large fragment from the chymosin gene as the target sequence, the usefulness of these vectors for studying foreign gene **expression** in streptococci as well as Escherichia **coli** was demonstrated.

L17 ANSWER 16 OF 71 MEDLINE on STN
ACCESSION NUMBER: 84221999 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6374659
TITLE: **Streptokinase**: **cloning**, **expression**, and excretion by Escherichia **coli**.
AUTHOR: Malke H; Ferretti J J
CONTRACT NUMBER: AI 9304 (NIAID)
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1984 Jun) 81 (11) 3557-61.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 198407
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19970203
Entered Medline: 19840724

AB Genomic DNA from Streptococcus **equisimilis** strain H46A was **cloned** in Escherichia **coli** by using the bacteriophage lambda replacement vector L47 and an in vitro packaging system. A casein/plasminogen overlay technique was used to screen the phage bank for **recombinants** carrying the **streptokinase** gene (**skc**). The gene was present with a frequency of 1 in 836 **recombinants**, and 10 independent **clones** containing **skc** were isolated and physically characterized. One **recombinant clone** was used to subclone **skc** in E. **coli** plasmid vectors. Plasmid pMF2 [10.4 kilobases (kb)] consisting of pACYC184 with a 6.4-kb H46A DNA fragment in the EcoRI site and pMF5 (6.9 kb) carrying a 2.5-kb fragment in the Pst I site of pBR322 were among the **recombinant** plasmids determining **streptokinase** production in three different E. **coli** host strains. **Expression** of **skc** was independent of its orientation in either vector, indicating that its own promoter was present and functional in E. **coli**. However, **expression** in pBR322 was more efficient in one orientation than in the other, suggesting that one or both of the bla gene promoters contributed to **skc expression**. Several lines of evidence, including proof obtained by the immunodiffusion technique, established the identity of E. **coli streptokinase**. Testing cell-free culture supernatant fluids, osmotic shock fluids, and sonicates of osmotically shocked cells for **streptokinase** activity revealed the substance to be present in all three principal locations, indicating that E. **coli** cells were capable of releasing substantial amounts of **streptokinase** into the culture medium.

L17 ANSWER 17 OF 71 EMBASE
ACCESSION NUMBER: 2005441717 EMBASE
TITLE: Heberkinasa: **Recombinant streptokinase** [9].
AUTHOR: Hernandez L.; Martinez Y.; Quintana M.; Besada V.; Martinez E.
CORPORATE SOURCE: L. Hernandez, Production Division, Centro de Ingenieria Genetica Y Biotecnologia, Ave 31 e/ 158 y 190, Cubanacan, Playa, Habana 0600, Cuba. luciano.hernandez@cigb.edu.cu
SOURCE: European Heart Journal, (2005) Vol. 26, No. 16, pp. 1691. Refs: 4
ISSN: 0195-668X CODEN: EHJODF
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Letter
FILE SEGMENT: 018 Cardiovascular Diseases and Cardiovascular Surgery
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
ENTRY DATE: Entered STN: 20051020
Last Updated on STN: 20051020

L17 ANSWER 18 OF 71 EMBASE
ACCESSION NUMBER: 2002110457 EMBASE
TITLE: Can imperfections help to improve bioreactor performance?.
AUTHOR: Patnaik P.R.
CORPORATE SOURCE: P.R. Patnaik, Institute of Microbial Technology, Sector 39-A, Chandigarh-160 036, India. pratap@imtech.res.in
SOURCE: Trends in Biotechnology, (1 Apr 2002) Vol. 20, No. 4, pp. 135-137.

Refs: 22
ISSN: 0167-7799 CODEN: TRBIDM
PUBLISHER IDENT.: S 0167-7799(01)01922-9
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
027 Biophysics, Bioengineering and Medical Instrumentation
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20020404
Last Updated on STN: 20020404

AB Pilot-scale and larger bioreactors differ from small laboratory-scale reactors in terms of a greater occurrence of noise and incomplete mixing of the broth. Conventional control tries to induce good mixing and to filter out the noise as completely as possible. As such an 'ideal' operation is difficult to achieve, recent work has tried to exploit the non-ideal features to improve the performance. Using artificial neural networks, the degree of mixing, the extent of filtering of noise and the distribution of plasmid copy number (in a **recombinant** fermentation) can be controlled effectively on-line. This strategy generates better productivities than well-mixed noise-free operations, which suggests that deviations from ideal behaviour should be gainfully harnessed and not suppressed.

L17 ANSWER 19 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:96119 BIOSIS
DOCUMENT NUMBER: PREV200000096119
TITLE: Two **streptokinase** genes are **expressed** with different solubility in Escherichia **coli** W3110.
AUTHOR(S): Pupo, Elder [Reprint author]; Baghbaderani, Behnam A.; Lugo, Victoria; Fernandez, Julio; Paez, Rolando; Torrens, Isis
CORPORATE SOURCE: Biopharmaceutical Development Division, Center for Genetic Engineering and Biotechnology, Havana, Cuba
SOURCE: Biotechnology Letters, (Dec., 1999) Vol. 21, No. 12, pp. 1119-1123. print.
CODEN: BILED3. ISSN: 0141-5492.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 15 Mar 2000
Last Updated on STN: 3 Jan 2002

AB The **streptokinase** (SK) gene from S. **equisimilis** H46A (ATCC 12449) was **cloned** in E. **coli** W3110 under the control of the tryptophan promoter. The **recombinant** SK, which represented 15% of total cell protein content, was found in the soluble fraction of disrupted cells. The solubility of this SK notably differed from that of the product of the SK gene from S. **equisimilis** (ATCC 9542) which had been **cloned** in E. **coli** W3110 by using similar **expression** vector and cell growth conditions, and occurred in the form of inclusion bodies.

L17 ANSWER 20 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:88244 BIOSIS
DOCUMENT NUMBER: PREV200000088244
TITLE: Hydrodynamic radii of native and denatured proteins measured by pulse field gradient NMR techniques.
AUTHOR(S): Wilkins, Deborah K.; Grimshaw, Shaun B.; Receveur, Veronique; Dobson, Christopher M.; Jones, Jonathan A.; Smith, Lorna J. [Reprint author]
CORPORATE SOURCE: Oxford Centre for Molecular Sciences, New Chemistry

Laboratory, University of Oxford, South Parks Road, Oxford, OX1 3QT, UK
SOURCE: Biochemistry, (Dec. 14, 1999) Vol. 38, No. 50, pp. 16424-16431. print.
CODEN: BICHAW. ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Mar 2000
Last Updated on STN: 3 Jan 2002

AB Pulse field gradient NMR methods have been used to determine the effective hydrodynamic radii of a range of native and nonnative protein conformations. From these experimental data, empirical relationships between the measured hydrodynamic radius (Rh) and the number of residues in the polypeptide chain (N) have been established; for native folded proteins Rh = 4.75N 0.29ANG and for highly denatured states Rh = 2.21N 0.57ANG. Predictions from these equations agree well with experimental data from dynamic light scattering and small-angle X-ray or neutron scattering studies reported in the literature for proteins ranging in size from 58 to 760 amino acid residues. The predicted values of the hydrodynamic radii provide a framework that can be used to analyze the conformational properties of a range of nonnative states of proteins. Several examples are given here to illustrate this approach including data for partially structured molten globule states and for proteins that are unfolded but biologically active under physiological conditions. These reveal evidence for significant coupling between local and global features of the conformational ensembles adopted in such states. In particular, the effective dimensions of the polypeptide chain are found to depend significantly on the level of persistence of regions of secondary structure or features such as hydrophobic clusters within a conformational ensemble.

L17 ANSWER 21 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1998:317596 BIOSIS
DOCUMENT NUMBER: PREV199800317596
TITLE: Effect of signal peptide changes on the extracellular processing of **streptokinase** from Escherichia **coli**: Requirement for secondary structure at the cleavage junction.
AUTHOR(S): Pratap, J.; Dikshit, K. L. [Reprint author]
CORPORATE SOURCE: Inst. Microbial Technology, Sector 39-A, Chandigarh 160 036, India
SOURCE: Molecular and General Genetics, (May, 1998) Vol. 258, No. 4, pp. 326-333. print.
CODEN: MGGEAE. ISSN: 0026-8925.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 22 Jul 1998
Last Updated on STN: 10 Sep 1998

AB **Streptokinase** (SK), an extracellular protein from Streptococcus **equisimilis**, is secreted post-translationally by Escherichia **coli** using both its native and E. **coli**-derived transport signals. In this communication we report that cleavage specificity of signal peptidase I, and thus efficiency of secretion, varies in E. **coli** when SK export is directed by different transport signals. The native (+ 1) N-terminus of mature SK was retained when it was transported under the control of its own, PelB or LamB signal peptide. However, when translocation of SK was controlled by the OmpA or MalE signal peptide, Ala2 of mature SK was preferred as a cleavage site for the pre-SK processing. Our results indicate that compatibility of the leader peptide with the mature sequences of SK, which fulfils the requirement for a given secondary structure within the cleavage region, is essential for maintaining the correct processing of pre-SK. An OmpA-SK fusion, which results in the deletion of two N-terminal amino acid residues of mature

SK, was further studied with respect to the recognition of alternative cleavage site in E. **coli**. The alanine at +2 in mature SK was changed to glycine or its relative position was changed to +3 by introducing a methionine residue at the +1 position. Both alterations resulted in the correct cleavage of pre-SK at the original OmpA fusion site. In contrast, introduction of an additional alanine at +4, creating three probable cleavage sites (Ala-x-Ala-x-Ala-x-Ala), resulted in the recognition of all three target sites for cleavage, with varying efficiency. The results indicate that the nature of the secondary structure generated at the cleavage junction of pre-SK, resulting from the fusion of different signal peptides, modulates the cleavage specificity of signal peptidase I during extracellular processing of SK. Based on these findings it is proposed that flexibility in the interaction of the active site of signal peptidase I with the cleavage sites of signal peptides may occur when it encounters two or more juxtaposed cleavage sites. Preference for one cleavage site over another, then, may depend on fulfillment of secondary structure requirements in the vicinity of the pre-protein cleavage junction.

L17 ANSWER 22 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1995:547506 BIOSIS
DOCUMENT NUMBER: PREV199698561806
TITLE: High-level **expression** and secretion of **streptokinase** in Escherichia **coli**.
AUTHOR(S): Ko, Jae Hyeong; Park, Do Deun; Kim, Il Chul; Lee, Si Hyoung; Byun, Si Myung [Reprint author]
CORPORATE SOURCE: Dep. BioSci., Korea Advanced Inst. Sci. Technol., 373-1, Kusung-dong, Yusung-ku, Taejeon 305-701, South Korea
SOURCE: Biotechnology Letters, (1995) Vol. 17, No. 10, pp. 1019-1024.
CODEN: BILED3. ISSN: 0141-5492.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 31 Dec 1995
Last Updated on STN: 31 Dec 1995

AB The high-level **expression** plasmid for **streptokinase**, pSK100, has been constructed. It contains a tac promoter, an ompA signal sequence, a **streptokinase** structural gene(**skc**) and a rrnBT1T2 transcription terminator. E. **coli** JM 109 carrying pSK100 produced about 5,000IU of **streptokinase** per 1 ml of LB-ampicillin media. About 95% of the **expressed** **streptokinase** was secreted into the periplasmic and extracellular fractions. The **recombinant** **streptokinase** in high yield and purity may be a potential alternative source for the therapeutic agent.

L17 ANSWER 23 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1992:340953 BIOSIS
DOCUMENT NUMBER: PREV199243030503; BR43:30503
TITLE: **STREPTOKINASE** MUTATIONS AFFECTING **SKC** **EXPRESSION** IN HOMOLOGOUS AND HETEROLOGOUS HOSTS.
AUTHOR(S): MECHOLD U [Reprint author]; MULLER J; MALKE H
CORPORATE SOURCE: CENTRAL INST MICROBIOL EXP THERAPY, JENA D-6900, GER
SOURCE: Zentralblatt fuer Bakteriologie Supplement, (1992) pp. 336-338. OREFICI, G. (ED.). ZENTRALBLATT FUER BAKTERIOLOGIE SUPPLEMENT, 22. NEW PERSPECTIVES ON STREPTOCOCCI AND STREPTOCOCCAL INFECTIONS; (INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY, 22. NEW PERSPECTIVES ON STREPTOCOCCI AND STREPTOCOCCAL INFECTIONS); XI LANCEFIELD INTERNATIONAL SYMPOSIUM ON STREPTOCOCCI AND STREPTOCOCCAL DISEASES, SIENA, ITALY, SEPTEMBER 10-14, 1990. XIX+569P. GUSTAV FISCHER VERLAG: STUTTGART, GERMANY; NEW YORK, NEW YORK,

USA. ILLUS.
Publisher: Series: Zentralblatt fuer Bakteriologie Supplement.
ISSN: 0941-018X. ISBN: 3-437-11362-3, 1-56081-333-4.
DOCUMENT TYPE: Book
Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 16 Jul 1992
Last Updated on STN: 16 Jul 1992

L17 ANSWER 24 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1991:513401 BIOSIS
DOCUMENT NUMBER: PREV199141114116; BR41:114116
TITLE: **EXPRESSION** AND PROPERTIES OF HYBRID **STREPTOKINASES** EXTENDED BY AMINO-TERMINAL PLASMINOGEN KRINGLE DOMAINS.
AUTHOR(S): MALKE H [Reprint author]; FERRETTI J J
CORPORATE SOURCE: DEP MICROBIOL IMMUNOL, UNIV OKLA HEALTH SCI CENTER, OKLAHOMA CITY, OKLA 73190, USA
SOURCE: (1991) pp. 184-189. DUNNY, G. M., P. P. CLEARY AND L. L. MCKAY (ED.). GENETICS AND MOLECULAR BIOLOGY OF STREPTOCOCCI, LACTOCOCCI, AND ENTEROCOCCI; THIRD INTERNATIONAL ASM (AMERICAN SOCIETY FOR MICROBIOLOGY) CONFERENCE, MINNEAPOLIS, MINNESOTA, USA, JUNE 6-9, 1990. VIII+310P. AMERICAN SOCIETY FOR MICROBIOLOGY: WASHINGTON, D.C., USA. ILLUS.
ISBN: 1-55581-034-9.
DOCUMENT TYPE: Book
Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 14 Nov 1991
Last Updated on STN: 14 Nov 1991

L17 ANSWER 25 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1987:499919 BIOSIS
DOCUMENT NUMBER: PREV198733127633; BR33:127633
TITLE: **STREPTOKINASE EXPRESSION** OF ALTERED FORMS.
AUTHOR(S): MALKE H [Reprint author]; LORENZ D; FERRETTI J J
CORPORATE SOURCE: ACAD SCI GER DEMOCRATIC REPUBLIC, CENT INST MICROBIOL AND EXP THERAPY, DDR-69 JENA, GDR
SOURCE: (1987) pp. 143-149. FERRETTI, J. J. AND R. CURTISS, III (ED.). STREPTOCOCCAL GENETICS; SECOND ASM (AMERICAN SOCIETY FOR MICROBIOLOGY) CONFERENCE, MIAMI, FLORIDA, USA, MAY 21-24, 1986. VIII+300P. AMERICAN SOCIETY FOR MICROBIOLOGY: WASHINGTON, D.C., USA. ILLUS.
ISBN: 0-914826-93-X.
DOCUMENT TYPE: Book
Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 27 Nov 1987
Last Updated on STN: 27 Nov 1987

L17 ANSWER 26 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1987:277792 BIOSIS
DOCUMENT NUMBER: PREV198784018831; BA84:18831
TITLE: MOLECULAR **CLONING** OF **STREPTOKINASE** GENE FROM STREPTOCOCCUS-**EQUISIMILIS** AND ITS

EXPRESSION IN ESCHERICHIA-COLI.
AUTHOR(S): ROH D C [Reprint author]; KIM J H; PARK S K; LEE J W; BYRUN S M
CORPORATE SOURCE: DEP BIOLOGICAL SCIENCE AND ENGINEERING, KOREA ADVANCED INST SCIENCE AND TECHNOLOGY KAIST , PO BOX 150 CHONGRYANG, SEOUL 131, KOREA
SOURCE: Korean Biochemical Journal, (1986) Vol. 19, No. 4, pp. 391-398.
CODEN: KBCJAK. ISSN: 0368-4881.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 19 Jun 1987
Last Updated on STN: 19 Jun 1987

AB The streptococcal genomic DNA digested with Pst I was **cloned** in E. **coli** HB101. The overlay technique of casein/plasminogen was used to screen the **clones** for **recombinants** carrying the **streptokinase** gene. The insert size of the plasmid carrying the **streptokinase** gene was a 2.5, 4.3, and 5.8 Kb, respectively. The restriction maps of all three hybrid plasmids were constructed by digestion with Pst I, Pvu II, Sal I, Hind III, Ava I, BamH I, and Cla I. For the identification of **cloned** gene, **streptokinase** was highly purified from S. **equisimilis** by the methods of gel chromatography and isoelectric focusing and rabbits were immunized with this purified **streptokinase**. Several lines of evidence, including proof obtained by the immunodiffusion technique, established that the enzyme from E. **coli** was identical to that from S. **equisimilis**. In the E. **coli** cell culture, we found the activity of **streptokinase** in all three principal locations of the cell. More than 50% were existed in the intracellular space.

L17 ANSWER 27 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1984:100214 BIOSIS
DOCUMENT NUMBER: PREV198427016706; BR27:16706
TITLE: HYBRIDIZATION OF A **CLONED** GROUP C STREPTOCOCCAL STREPTO KINASE GENE WITH DNA FROM OTHER STREPTOCOCCAL SPECIES.
AUTHOR(S): HUANG T-T [Reprint author]; WEEKS C R; MALKE H; FERRETTI J J
CORPORATE SOURCE: UNIV OKLA HEALTH SCI CENT, OKLAHOMA CITY, OKLA, USA
SOURCE: Abstracts of the Annual Meeting of the American Society for Microbiology, (1984) Vol. 84, pp. ABSTRACT D75.
Meeting Info.: 84TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, ST. LOUIS, MO., USA, MAR. 4-9, 1984.
ABSTR ANNU MEET AM SOC MICROBIOL.
CODEN: ASMACK. ISSN: 0094-8519.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH

L17 ANSWER 28 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1984:92832 BIOSIS
DOCUMENT NUMBER: PREV198427009324; BR27:9324
TITLE: STREPTO KINASE **CLONING EXPRESSION** AND EXCRETION BY ESCHERICHIA-**COLI**.
AUTHOR(S): MALKE H [Reprint author]; FERRETTI J J
CORPORATE SOURCE: ACAD SCI GDR, JENA, GDR
SOURCE: Abstracts of the Annual Meeting of the American Society for Microbiology, (1984) Vol. 84, pp. ABSTRACT D97.
Meeting Info.: 84TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, ST. LOUIS, MO., USA, MAR. 4-9, 1984.
ABSTR ANNU MEET AM SOC MICROBIOL.

CODEN: ASMACK. ISSN: 0094-8519.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH

L17 ANSWER 29 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1994-07329 BIOTECHDS
TITLE: DNA encoding a plasminogen binding protein; **recombinant streptokinase** fragment production using new vector plasmid pMAL and a monoclonal antibody for use in myocardial infarction therapy
PATENT ASSIGNEE: Gen.Hosp.Boston; Univ.Harvard
PATENT INFO: WO 9407992 14 Apr 1994
APPLICATION INFO: WO 1993-US9502 5 Oct 1993
PRIORITY INFO: US 1993-128299 29 Sep 1993; US 1992-956692 5 Oct 1992
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1994-135561 [16]
AB DNA (I) encoding a **streptokinase** fragment (II) of residues 14-414 of a disclosed protein sequence is claimed. (II) does not contain residues 244-352, but may contain residues 1-352, 120-352, 244-414 or 244-352 of the protein sequence. Also claimed are: (1) an **expression** vector containing (I); (2) a host cell transformed with the vector of (1); (3) (II) encoded by (I); (4) detecting plasminogen in a biological sample by contacting the sample with (II) and detecting any (II)-plasminogen complex formed; (5) a method for assaying (II) for antigenicity involving contacting (II) with a monoclonal antibody specific for a distinct epitope of **streptokinase** and determining whether the fragments bind to the MAb and, optionally, also whether the fragments can activate plasminogen in the presence of the MAb; (6) a method for myocardial infarction therapy involving administering (II) to a patient; and (7) a monoclonal antibody specific for a distinct epitope of **streptokinase**. In an example, (II) genes from Streptococcus **equisimilis** were fused with maltose binding protein genes and **expressed** in Escherichia **coli** using plasmid pMAL. (62pp)

L17 ANSWER 30 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1992-13545 BIOTECHDS
TITLE: High level **expression** of **streptokinase** in Escherichia **coli**; gene **cloning**, **expression** and purification of thrombolytic protein
AUTHOR: Estrada M P; Hernandez L; Perez A; Rodriguez P; *de la Fuente J; Herrera L
LOCATION: Mammalian Cell Genetics Division, Centro de Ingenieria Genetica y Biotecnologia, P.O. Box 6162, Havana 6, Cuba.
SOURCE: Bio/Technology; (1992) 10, 10, 1138-42
CODEN: BTCHDA
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **streptokinase** (SK) gene was isolated by the polymerase chain reaction from Streptomyces **equisimilis** ATCC 9542. The 5-amplification primer introduced an ATG codon for translation initiation in Escherichia **coli**. The amplified fragment, which lacked the signal peptide sequence, was digested with BamHI, inserted into vector plasmid pTrp (to obtain plasmid pEKG-3 containing the SK gene under the control of a trp promoter), and used to transform E. **coli** HB101 cells. The DNA sequence of the SK gene region contained 5 differences at the amino acid level with respect to the reported SK protein. Plasmid pEKG-3 was introduced into E. **coli** K-12 strain W3110 for **expression**. The trp promoter was induced, and maximal SK **expression** was obtained after 14 hr, at which time the plasmid copy number reached 420 copies/cell. The **recombinant** SK was

found in the cell cytosol and constituted 25% of total cell protein. It was purified by affinity chromatography using acylated human plasminogen coupled to Sepharose-4B, and ionexchange chromatography on DEAE-Sephacel. The **recombinant** product and natural SK had equivalent biological activities. (38 ref)

L17 ANSWER 31 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1992-03808 BIOTECHDS
TITLE: Method for the isolation and **expression** of a gene encoding **streptokinase**;
Streptococcus **equisimilis** gene **cloning** and vector plasmid pEKG3, plasmid pPESKC-4 and plasmid pPISKC-6 **expression** in Escherichia **coli** or Pichia pastoris
PATENT ASSIGNEE: Cent.Ing.Genet.Biotecnol.
PATENT INFO: AU 9178101 28 Nov 1991
APPLICATION INFO: AU 1991-78101 31 May 1991
PRIORITY INFO: CU 1990-90 23 May 1990
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1992-024716 [04]
AB A new method for the isolation and **expression** of a gene, **SKC-2**, encoding Streptococcus **equisimilis** C (ATCC 9542) **streptokinase** comprises (i) gene amplification from synthetic oligonucleotides SK1, SK2 and SK3 (specified DNA sequence), (ii) **cloning SKC-2** in bacteria (preferably Escherichia **coli**) with or without a signal peptide; and (iii) intra- or extracellular **expression** in yeast (preferably Pichia pastoris), with the transformed microorganism displaying a high stability and level of **expression**. The following are also claimed: (1) plasmid pEKG3 containing **SKC-2** inserted between the trp promoter and the phage T4 terminator for **expression** in bacteria; (2) plasmid pPESKC-4 and plasmid pPISKC-6, obtained by insertion of **SKC-2** in the yeast **expression** vectors plasmid pPS-7 and plasmid pNAO, respectively, for extra- or intracellular **expression**; (3) transformed microorganisms displaying high levels of **SKC-2** gene **expression**, good viability and cellular stability; (4) the product resulting from **expression** of the **SKC-2** gene in bacteria and yeast; (5) **recombinant** DNA comprising the **SKC-2** DNA sequence; and (6) the **expression** product of the **recombinant** DNA. (28pp)

L17 ANSWER 32 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1991-01878 BIOTECHDS
TITLE: Constructing vector for detecting **expression** of foreign genes;
by inserting element containing **expression** unit, **streptokinase** gene and restriction sites, allowing in frame gene insertion; pro-chymosin, beta-galactosidase production
PATENT ASSIGNEE: Akad.Wiss.DDR
PATENT INFO: DD 279900 20 Jun 1990
APPLICATION INFO: DD 1987-306609 3 Sep 1987
PRIORITY INFO: DD 1987-306609 3 Sep 1987
DOCUMENT TYPE: Patent
LANGUAGE: German
OTHER SOURCE: WPI: 1990-342373 [46]
AB Construction of vectors for detecting heterologous gene **expression** comprises: i. incorporating (in order from N terminus) into a **cloning** vector, a polylinker or restriction site (RS1); **expression** or **expression** secretion unit (Escherichia **coli** lac operon or exotoxin A gene of phage T12 from Streptococcus pyogenes; polylinker or restriction site (RS2);

streptokinase (SK) structural gene (from Streptococcus **equisimilis** H46A, particularly a 1596 bp HindIII fragment from plasmid pMF5), a polylinker or restriction site (RS3); ii. inserting a foreign gene, X, without a promoter into polylinker or RS2; iii. the resulting detection vector, encoding for an X-SK fusion product, is used to transform microbial receptor cells; and iv. subjecting **recombinant clones** to a plasminogen-milk agar (PMA) overlaying test. Preferably, the vector is a bacterial plasmid or an M13 E. **coli** phage vector. X is a pro-chymosin gene (plasmid pHRW400 or plasmid pHRW500), human interferon-alpha-1, or beta-galactosidase (EC-3.2.1.23, from a pUC plasmid). Recipients are E. **coli** JM101, Streptococcus sanguis Challis 6 or Streptococcus lactis MG1363. (14pp)

L17 ANSWER 33 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1990-02600 BIOTECHDS
TITLE: Site-specific alteration of Gly-24 in **streptokinase** : its effect on plasminogen activation; site-directed mutagenesis effect on plasminogen-activator activity; gene **cloning** and **expression** in Escherichia **coli**
AUTHOR: Lee B R; Park S K; Kim J H; *Byun S M
LOCATION: Department of Biological Science and Engineering, Korea Advanced Institute of Science and Technology (KAIST), P.O. Box 150, Cheongryang, Seoul, Korea.
SOURCE: Biochem.Biophys.Res.Commun.; (1989) 165, 3, 1085-90 CODEN: BBRCA9
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Oligonucleotide site-directed mutagenesis was performed to replace Gly-24 of Streptococcus **equisimilis** (ATCC 9542) **streptokinase** with His, Glu, or Ala. The **streptokinase** gene was **cloned**, subjected to mutagenesis for removal of the RsaI site, **cloned** into vector plasmid pKS601 under the control of the trp promoter and used to transform Escherichia **coli** C600. The **recombinant** proteins were purified by DEAE-cellulose and Sephadex-G150 chromatography. Substitutions with either His or Glu gave almost complete loss of **streptokinase** activity but **streptokinase** replaced with Ala retained its activity. Although **streptokinases** with His-24 or Glu-24 bound normally to human plasminogen, they did not generate active plasmin, whereas those with Ala-24 or Gly-24 generated active plasmin. The results indicate that the small, uncharged alkyl group side chain on the 24th amino acid residue of **streptokinase** is indispensable for the activity of the human plasminogen-**streptokinase** complex. A charged amino acid in position 24 disrupts beta-sheet formation and prevents **streptokinase** from adopting the orientation required for plasminogen activation. (26 ref)

L17 ANSWER 34 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1985-08513 BIOTECHDS
TITLE: Production of **streptokinase**; by cultivation of Escherichia **coli** ATCC 39613 containing **recombinant** plasmid PMF1
PATENT ASSIGNEE: Phillips-Petrol.
PATENT INFO: AU 8433859 18 Apr 1985
APPLICATION INFO: AU 1984-33859 5 Oct 1984
PRIORITY INFO: US 1984-585417 2 Mar 1984; DD 1983-255523 10 Oct 1983
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1985-135032 [23]
AB A **recombinant** vector plasmid pMF1 for the transformation of a host to produce **streptokinase** is new. The vector contains a polydeoxyribonucleotide fragment insert which codes for the synthesis and

secretion of **streptokinase**. The transformant microorganism is preferably Escherichia **coli** HB101 and the vector, plasmid pBR322. The fragment coding for **streptokinase** synthesis and secretion is derived from a microorganism of the genus Streptococcus, especially Streptococcus **equisimilis** strain H46A and may have restriction endonuclease cleavage sites at the terminii e.g. it has 7400 bp and the cleavage sites are for HindIII. The **recombinant** vector is obtained by digestion of a vector with a restriction endonuclease to give linear DNA. This DNA is ligated to the **streptokinase** fragment to give the **recombinant**. This fragment is obtained by digestion of Streptococcus equilimilis with the same restriction endonuclease, especially Pst I, as is used to digest the initial vector. **Streptokinase** can be produced and isolated for use as a thrombolytic agent to facilitate the in vivo lysis or dissolution of blood clots. (28pp)

L17 ANSWER 35 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1984-03868 BIOTECHDS
TITLE: **Streptokinase: cloning expression** and excretion by E.**coli**; using Streptococcus **equisimilis** genomic DNA (conference abstract)
AUTHOR: Malke H; Ferretti J J
LOCATION: Acad. Sci. GDR, Jena, DDR.
SOURCE: Abstr.Annu.Meet.Am.Soc.Microbiol; (1984) 84 Meet., 67
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Genomic DNA from Streptococcus **equisimilis** H46A (group C) was **cloned** into Escherichia **coli** using the lambda replacement vector L47 and an in vitro packaging system. The phage bank was screened for **recombinants** containing the **streptokinase** (**skc**) gene by the casein-plasminogen overlay technique. 10 Independent **clones** containing the **skc** gene were isolated and one was used to subclone the **skc** gene into E.**coli** plasmid vectors pBR322 and pACYC184. Plasmid pMF2 and pMF5 were among the **recombinant** plasmids determining **streptokinase** production in 3 different E. **coli** host strains. **Expression** of **skc** was independent of its orientation in either vector, indicating that its own promoter was present and functional in E.**coli**. Analysis of cell free culture supernatant fluids, osmotic shockates, and sonicates of osmotically shocked cells for **streptokinase** activity revealed the substance to be present in all 3 locations, indicating that E. **coli** cells were capable of releasing substantial amounts of **streptokinase** into the culture medium. (0 ref)

L17 ANSWER 36 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:656911 SCISEARCH
THE GENUINE ARTICLE: 837ZE
TITLE: Control of **streptokinase** gene **expression** in group A & C streptococci by two-component regulators
AUTHOR: Malke H (Reprint); Steiner K
CORPORATE SOURCE: Univ Jena, Inst Mol Biol, Winzerlaer Str 10, D-07745 Jena, Germany (Reprint); Univ Jena, Inst Mol Biol, D-07745 Jena, Germany
hmalke@imb-jena.de
COUNTRY OF AUTHOR: Germany
SOURCE: INDIAN JOURNAL OF MEDICAL RESEARCH, (MAY 2004) Vol. 119, Supp. [S], pp. 48-56.
ISSN: 0971-5916.
PUBLISHER: INDIAN COUNCIL MEDICAL RES, PO BOX 4911 ANSARI NAGAR, NEW DELHI 110029, INDIA.
DOCUMENT TYPE: Article; Journal

LANGUAGE: English
REFERENCE COUNT: 23
ENTRY DATE: Entered STN: 13 Aug 2004
Last Updated on STN: 13 Aug 2004
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background & objectives: Group A streptococci (GAS) and human isolates of group C streptococci (GCS) have the stable capacity to produce the plasminogen activator **streptokinase**, albeit with varying efficiency. This property is subject to control by two two-component regulatory systems, FasCAX and CovRS, which act as activator and repressor, respectively. The present work aims at balancing these opposing activities in GAS and GCS, and at clarifying the phylogenetic position of the FasA response regulator, the less understood regulator of the two systems.

Methods: The GCS strain H46A and GAS strain NZ131 were used. Escherichia **coli** JM 109 was used as host for plasmid construction. **Streptokinase** activity of various wild type and mutant strains was measured. Phylogenetic trees of streptococcal FasA homologues were established.

Results: The **streptokinase** activities of the GAS strain NZ131 and the GCS strain H46A were attributable to more efficient CovR repressor action in NZ131 than in H46A. The FasA activator, on the other hand, functioned about equally efficient in the two strains. Phylogenetically, FasA homologues clustered distinctly in the proposed FasA-BlpR-ComE family of streptococcal response regulators and used the LytTR domain for DNA binding.

Interpretation & conclusion: Assessing the apparent **streptokinase** activity of streptoccal strains require the dissection of the activities of the cov and fas systems. Although experimental evidence is still missing, FasA is closely related to a widely distributed family of streptococcal response regulators that is involved in behavioral processes, such as quorum sensing.

L17 ANSWER 37 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:69727 SCISEARCH
THE GENUINE ARTICLE: 763KK
TITLE: **Streptokinase** - a clinically useful thrombolytic agent
AUTHOR: Banerjee A; Chisti Y; Banerjee U C (Reprint)
CORPORATE SOURCE: Natl Inst Pharmaceut Educ & Res, Dept Biotechnol, Sector 67, Mohali 160062, Punjab, India (Reprint); Natl Inst Pharmaceut Educ & Res, Dept Biotechnol, Mohali 160062, Punjab, India; Massey Univ, Inst Technol & Engn, Palmerston North, New Zealand
COUNTRY OF AUTHOR: India; New Zealand
SOURCE: BIOTECHNOLOGY ADVANCES, (FEB 2004) Vol. 22, No. 4, pp. 287-307.
ISSN: 0734-9750.
PUBLISHER: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND.
DOCUMENT TYPE: General Review; Journal
LANGUAGE: English
REFERENCE COUNT: 171
ENTRY DATE: Entered STN: 30 Jan 2004
Last Updated on STN: 30 Jan 2004
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A failure of hemostasis and consequent formation of blood clots in the circulatory system can produce severe outcomes such as stroke and myocardial infraction. Pathological development of blood clots requires clinical intervention with fibrinolytic agents such as urokinase, tissue plasminogen activator and **streptokinase**. This review deals with **streptokinase** as a clinically important and cost-effective plasminogen activator. The aspects discussed include: the mode of action;

the structure and structure-function relationships; the structural modifications for improving functionality; **recombinant streptokinase**; microbial production; and recovery of this protein from crude broths. (C) 2003 Published by Elsevier Inc.

L17 ANSWER 38 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:148580 SCISEARCH
THE GENUINE ARTICLE: 285UW
TITLE: Allele substitution of the **streptokinase** gene reduces the nephritogenic capacity of group A streptococcal strain NZ131
AUTHOR: Nordstrand A (Reprint); McShan W M; Ferretti J J; Holm S E; Norgren M
CORPORATE SOURCE: Umea Univ, Dept Clin Bacteriol, S-90185 Umea, Sweden (Reprint); Univ Oklahoma, Hlth Sci Ctr, Dept Microbiol & Immunol, Oklahoma City, OK 73190 USA
COUNTRY OF AUTHOR: Sweden; USA
SOURCE: INFECTION AND IMMUNITY, (MAR 2000) Vol. 68, No. 3, pp. 1019-1025.
ISSN: 0019-9567.
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 36
ENTRY DATE: Entered STN: 2000
Last Updated on STN: 2000

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB To investigate the role of allelic variants of **streptokinase** in the pathogenesis of acute poststreptococcal glomerulonephritis (APSGN), site-specific integration plasmids were constructed, which contained either the non-nephritis-associated **streptokinase** gene (skc5) from the group C streptococcal strain Streptococcus **equisimilis** H46A or the nephritis-associated **streptokinase** gene (ska1) from the group A streptococcal nephritogenic strain NZ131. The plasmids were introduced by electroporation and homologous recombination into the chromosome of an isogenic derivative of strain NZ131, in which the **streptokinase** gene had been deleted and which had thereby lost its nephritogenic capacity in a mouse model of APSGN. The introduction of a non-nephritis-associated allelic variant of **streptokinase** did not rescue the nephritogenic capacity of the strain. The mutant and the wild-type strains produced equivalent amounts of **streptokinase**. Complementation of the ska deletion derivative with the original ska allele reconstituted the nephritogenicity of wild-type NZ131. The findings support the hypothesis that the role of **streptokinase** in the pathogenesis of APSGN is related to the allelic variant of the protein.

L17 ANSWER 39 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:100732 SCISEARCH
THE GENUINE ARTICLE: 277JH
TITLE: Genetic organisation of the M protein region in human isolates of group C and G streptococci: two types of multigene regulator-like (mgrC) regions
AUTHOR: Geyer A; Schmidt K H (Reprint)
CORPORATE SOURCE: Univ Jena, Univ Hosp, Inst Med Microbiol, Semmelweissstr 4, D-07740 Jena, Germany (Reprint); Univ Jena, Univ Hosp, Inst Med Microbiol, D-07740 Jena, Germany
COUNTRY OF AUTHOR: Germany
SOURCE: MOLECULAR AND GENERAL GENETICS, (JAN 2000) Vol. 262, No. 6, pp. 965-976.
ISSN: 0026-8925.

PUBLISHER: SPRINGER-VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 47
ENTRY DATE: Entered STN: 2000
Last Updated on STN: 2000
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In addition to beta-haemolytic streptococci belonging to Lancefield group A (Streptococcus pyogenes, GAS), human isolates of group C (GCS) and group G (GGS) streptococci (S. dysgalactiae subsp. **equisimilis**:) have been implicated as causative agents in outbreaks of purulent pharyngitis, of wound infections and recently also of streptococcal toxic shock-like syndrome. Very little is known about the organisation of the genomic region in which the emm gene of GCS and GCS is located. We have investigated the genome sequences flanking the emm gene in GCS by sequencing neighbouring fragments obtained by inverse PCR. Our sequence data for GCS strains 25287 and H46A revealed two types of arrangement in the emm region, which differ significantly from the known types of mga regulon in GAS. We named this segment of the genome mgrC (for multigene regulon-like segment in group C streptococci). In strains belonging to the first mgrC type (prototype strain 25287) the emm gene is flanked upstream by mgc, a gene that is 61% identical to the mga gene of GAS. A phylogenetic analysis of the deduced protein sequences showed that Mgc is related to Mga proteins of various types of GAS but forms a distinct cluster. Downstream of emm, the mgrC sequence region is bordered by rel. This gene encodes a protein that functions in the synthesis and degradation of guanosine 3',5' bipyrophosphate (ppGpp) during the stringent regulatory response to amino acid deprivation. In the second mgrC type (prototype strain H46A), the genes mgc and emm are arranged as in type 1. But an additional ORF (orf) is inserted in opposite orientation between emm and rel. This orf shows sequence homology to cpdB, which is present in various microorganisms and encodes 2',3' cyclo-nucleotide 2'-phosphodiesterase. PCR analysis showed that these two mgrC arrangements also exist in GGS. Our sequence and PCR data further showed that both types of mgrC region in GCS and GGS are linked via rel to the **streptokinase** region characterised recently in strain H46A.
A gene encoding C5a peptidase, which is present at the 3' end of the mga regulon in GAS, was not found in the mgrC region identified in the GCS and GGS strains investigated here.

L17 ANSWER 40 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1999:666643 SCISEARCH
THE GENUINE ARTICLE: 231GM
TITLE: PauA: a novel plasminogen activator from Streptococcus uberis
AUTHOR: Rosey E L; Lincoln R A; Ward P N; Yancey R J; Leigh J A (Reprint)
CORPORATE SOURCE: Inst Anim Hlth, Compton Lab, Newbury RG20 7NN, Berks, England (Reprint); Pfizer Inc, Div Cent Res, Anim Hlth Biol Discovery, Groton, CT 06340 USA
COUNTRY OF AUTHOR: England; USA
SOURCE: FEMS MICROBIOLOGY LETTERS, (1 SEP 1999) Vol. 178, No. 1, pp. 27-33.
ISSN: 0378-1097.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 20
ENTRY DATE: Entered STN: 1999
Last Updated on STN: 1999
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Chromosomal DNA from two geographically distinct isolates of

Streptococcus uberis was used to **clone** the plasminogen activator in an active form in Escherichia **coli**. The **cloned** fragments from each strain contained four potential open reading frames (ORFs). That for the plasminogen activator encoded a protein of 286 amino acids (33.4 kDa) which is cleaved between residues 25 and 26 during secretion by S. uberis. The amino acid sequence of the mature protein showed only weak homology (23.5-28%) to **streptokinase**. The plasminogen activator gene, pauA, in S. uberis was located between two ORFs with high homology to the DNA mismatch repair genes, hexA and hexB, and not on a DNA fragment between the genes encoding an ATP binding cassette transporter protein (abc) and a protein involved in the formation and degradation of guanosine polyphosphates (rel) as is the case for **streptokinase** in other streptococci.

L17 ANSWER 41 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:317401 SCISEARCH
THE GENUINE ARTICLE: 189BZ

TITLE: Hydrophobic interaction chromatography applied to purification of **recombinant streptokinase**

AUTHOR: Perez N (Reprint); Urrutia E; Camino J; Orta D R; Torres Y; Martinez Y; Cruz M; Alburquerque S; Gil M R; Hernandez L

CORPORATE SOURCE: Ctr Genet Engn & Biotechnol, Streptokinase Div, Havana, Cuba; Ctr Genet Engn & Biotechnol, Qual Control Div, Havana, Cuba

COUNTRY OF AUTHOR: Cuba

SOURCE: MINERVA BIOTECNOLOGICA, (DEC 1998) Vol. 10, No. 4, pp. 174-177.
ISSN: 1120-4826.

PUBLISHER: EDIZIONI MINERVA MEDICA, CORSO BRAMANTE 83-85 INT JOURNALS DEPT., 10126 TURIN, ITALY.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 22

ENTRY DATE: Entered STN: 1999
Last Updated on STN: 1999

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background **Recombinant streptokinase** (rSk) is a streptococcal protein **cloned** in E. **coli**.(11) Several methods have been described for **streptokinase** purification: ion exchange chromatography,(12) affinity chromatography with canine plasmin(13) and chromatography on immobilized acylated human plasminogen.(14) Monoclonal antibodies anti-rSk immobilized to Sepharose(15) have been used too. Recently this protein was purified using HIC.

Methods. rSk (CIGB, Cuba) was produced by fermentation strain K12 of E. **coli**,(11) the protein was recovered after washed pellet, cellular disruption and solubilization. Several purification assays were done using TSK-Butyl (Tosohaas, Japan) as a support for hydrophobic interaction chromatography (HIC). The protein was loaded to the column with 1 M of ammonium sulfate before being washed using an elution gradient from 0.5 to 0 M of ammonium sulfate, in order to determine the elution point of the rSk.

Results. Wie could determine that this protein is partly hydrophobic, this determination was shown by analysis of its aminoacidic sequence. This protein has 415 aminoacids of which 36% are non polar. The absorption capacity for TSK Butyl 650 S varies from 15 to 20 mg/mL. The optimum elution point was obtained using 0.25 M of ammonium sulfate, the eluted material was obtained with a high level of purity (<1% of contaminants). The recovery of rSk was about 49% using the mean of five

assays.
Conclusions. The experimental process evaluated could be efficiently inserted in a downstream process to obtain **recombinant streptokinase** highly purified as final preparation and good recovery.

L17 ANSWER 42 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1998:473042 SCISEARCH
THE GENUINE ARTICLE: ZV077
TITLE: The interaction of Streptococcus dysgalactiae with plasmin and plasminogen
AUTHOR: Leigh J A (Reprint); Hodgkinson S M; Lincoln R A
CORPORATE SOURCE: Inst Anim Hlth, Compton Lab, Newbury RG20 7NN, Berks, England (Reprint)
COUNTRY OF AUTHOR: England
SOURCE: VETERINARY MICROBIOLOGY, (15 MAR 1998) Vol. 61, No. 1-2, pp. 121-135.
ISSN: 0378-1135.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 29
ENTRY DATE: Entered STN: 1998
Last Updated on STN: 1998
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The activation of plasminogen and the binding of plasmin by bacteria may have many effects which promote infection. The occurrence of such activities in streptococci is well documented; however, these are yet to be demonstrated for S. dysgalactiae. Consequently, the ability of this bacterium to activate mammalian plasminogen and bind either plasmin or its zymogen was investigated. Activation of bovine plasminogen was dependant on both the strain and the growth medium used for cultivation. Eighteen strains were able to activate bovine and ovine plasminogen and some of these also activated plasminogen from the horse, rabbit and pig. None activated human plasminogen and one strain (CE127) did not activate plasminogen from any source. Tricine-SDS PAGE and zymographic analysis of culture supernatants showed that bovine plasminogen was activated by four out of six strains at two locations corresponding to 16 kDa and IO kDa. Following the growth of five strains in the presence of bovine plasminogen, all but strain CE127 bound high levels of plasmin activity. In contrast, following growth in human plasminogen none of the strains exhibited bound plasmin activity although all could bind human plasmin directly. Ail strains were also able to bind bovine and human plasminogen in such a way as to allow its activation by urokinase. We conclude that S. dysgalactiae is capable of activating mammalian plasminogen in a species-specific fashion and that the bacterium is also capable of binding plasmin and plasminogen with an apparent preference for bovine plasmin over human plasmin and/or plasminogen from either species. (C) 1998 Elsevier Science B.V.

L17 ANSWER 43 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1998:437021 SCISEARCH
THE GENUINE ARTICLE: ZU066
TITLE: The Streptococcus agalactiae hylB gene encoding hyaluronate lyase: completion of the sequence and **expression** analysis
AUTHOR: Gase K; Ozegowski J; Malke H (Reprint)
CORPORATE SOURCE: Univ Jena, Inst Mol Biol, Winzerlaer Str 10, D-07745 Jena, Germany (Reprint); Univ Jena, Inst Mol Biol, D-07745 Jena, Germany; Univ Jena, Inst Expt Microbiol, D-07745 Jena, Germany

COUNTRY OF AUTHOR: Germany
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA-GENE STRUCTURE AND EXPRESSION, (29 MAY 1998) Vol. 1398, No. 1, pp. 86-98. ISSN: 0167-4781.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 43
ENTRY DATE: Entered STN: 1998
Last Updated on STN: 1998
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We report the **cloning**, sequencing and **expression** analysis of the Streptococcus agalactiae strain 4755 hylB(4755) allele, the first chromosomally-encoded streptococcal hyaluronate lyase gene to be **cloned** and sequenced completely. This gene lies in a region homologous to that found in S. mutans, between the mutX and rmlB genes, a region involved in the synthesis of the serotype c-specific polysaccharide antigen of this organism. Sequencing of hylB(4755) revealed a 3216-bp open reading frame that encodes a 121.2-kDa polypeptide possessing a 30-amino acid signal sequence which was theoretically predicted and experimentally confirmed. A **recombinant** plasmid, pHYB100, containing hylB(4755) together with its promoter and terminator was constructed and used to analyze the **expression** of the gene in Escherichia **coli**. In Northern hybridization experiments, hylB(4755) was found to be transcribed as 3.3-kb monocistronic mRNA from its own promoter which exhibits an extended, sigma(70)-like 10 consensus sequence. Transcript mapping by primer extension analysis placed the major transcription initiation site leading to the longest transcript 38 bp upstream of the translational initiation codon: ATG. E. **coli** TG1(pHYB100) efficiently synthesized hyaluronan-cleaving enzyme activity at similar to 7000 working units/10(9) cells, with lyase activity detectable in all principle cellular locations. Zymography and Western analysis identified functional activity in TG1(pHYB100) to be associated with similar to 118, 110 and 94-kDa polypeptides, with the two low molecular weight species constituting the major components of the enzyme purified from the culture supernatant fluid of S. agalactiae 4755. The 118-kDa form was shown to represent the undegraded mature enzyme, whereas the smaller species are likely to arise from proteolytic cleavage in the N-terminal part of the mature protein. The HylB(4755) protein showed extensive sequence identity to the homologous enzymes from S. agalactiae 3502 and S. pneumoniae characterized by others but sequence comparisons clearly show that incomplete genes truncated at their 5' ends had been isolated from these two organisms. (C) 1998 Elsevier Science B.V. All rights reserved.

L17 ANSWER 44 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1997:547160 SCISEARCH
THE GENUINE ARTICLE: XL484
TITLE: The LppC gene of Streptococcus **equisimilis** encodes a lipoprotein that is homologous to the e(P4) outer membrane protein from Haemophilus influenzae
AUTHOR: Gase K (Reprint); Liu G W; Bruckmann A; Steiner K; Ozegowski J; Malke H
CORPORATE SOURCE: UNIV JENA, INST MOL BIOL, D-07745 JENA, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: MEDICAL MICROBIOLOGY AND IMMUNOLOGY, (JUN 1997) Vol. 186, No. 1, pp. 63-73. ISSN: 0300-8584.
PUBLISHER: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English

REFERENCE COUNT: 39
ENTRY DATE: Entered STN: 1997
Last Updated on STN: 1997
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We report the **cloning**, sequencing, and analysis of a novel chromosomal gene of Streptococcus **equisimilis** strain H46A that codes for a membrane lipoprotein, designated LppC. The lppC gene is located 3' adjacent to, and co-oriented with, the unrelated gapC gene that encodes the previously characterized glyceraldehyde-3-phosphate dehydrogenase. Sequencing of lppC revealed an 855-bp open reading frame that predicted a 32.4-kDa polypeptide possessing a potential lipoprotein signal sequence and modification site (VTGC). Signal sequence processing of LppC synthesized in the homologous host or **expressed** from plasmid pLPP2 in Escherichia **coli** was sensitive to globomycin, a selective inhibitor of lipoprotein-specific signal peptidase II. Subcellular localization of LppC using polyclonal antibodies raised to the hexahistidyl-tagged protein proved LppC to be tightly associated with the cytoplasmic membrane of S. **equisimilis** and with the outer membrane of E. **coli** JM109 (pLPP2). Southern, Northern and Western analyses indicated that Ipl, was conserved in S. pyogenes, and transcribed independently of gap as monocistronic 0.9-kb mRNA from a sigma(70)-like consensus promoter. Database searches found homology of LppC to the hel gene-encoded outer membrane protein e (P4) from Haemophilus influenzae to which it exhibits 58% sequence similarity. However, unlike the hel gene, lppC was unable to complement hemA mutants of E. **coli** for growth on hemin as sole porphyrin source in aerobic conditions. Furthermore, neither the wild type nor an lppC insertion mutant of S. **equisimilis** could grow on hemin in iron-limited medium. These results, together with findings indicating that S. **equisimilis** H46A had no absolute requirement for iron, led us to conclude that lppC, in contrast to hel, is not involved in hemin utilization and has yet to be assigned a function.

L17 ANSWER 45 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1996:43901 SCISEARCH
THE GENUINE ARTICLE: TN446
TITLE: **Cloning** and sequencing of the **streptokinase** gene from Streptococcus pyogenes (CIP 56.57)
AUTHOR: Ball M M (Reprint); Puig J; Iborra F
CORPORATE SOURCE: UNIV PARIS 11, F-91405 ORSAY, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: DNA SEQUENCE, (1995) Vol. 6, No. 1, pp. 33-36. ISSN: 1042-5179.
PUBLISHER: HARWOOD ACAD PUBL GMBH, C/O STBS LTD, PO BOX 90, READING, BERKS, ENGLAND RG1 8JL.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 10
ENTRY DATE: Entered STN: 1996
Last Updated on STN: 1996
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The **streptokinase** gene of the Streptococcus pyogenes strain CIP 56.57 was **cloned** and sequenced. This sequence coding for a 441 amino acid protein is well conserved among streptococcus species: there are two very conserved domains separated by a more variable region.

L17 ANSWER 46 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1995:622543 SCISEARCH
THE GENUINE ARTICLE: RU780
TITLE: **STREPTOKINASE**-MEDIATED PLASMINOGEN ACTIVATION

USING A **RECOMBINANT** DUAL FUSION PROTEIN CONSTRUCT - A NOVEL-APPROACH TO STUDY BACTERIAL HOST PROTEIN INTERACTIONS

AUTHOR: LIZANO S (Reprint); JOHNSTON K H
CORPORATE SOURCE: LOUISIANA STATE UNIV, MED CTR, DEPT MICROBIOL IMMUNOL & PARASITOL, NEW ORLEANS, LA 70112
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF MICROBIOLOGICAL METHODS, (SEP 1995) Vol. 23, No. 3, pp. 261-280.
ISSN: 0167-7012.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 59
ENTRY DATE: Entered STN: 1995
Last Updated on STN: 1995
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Streptokinase** (SK), a plasminogen (Pg) activator secreted by groups A, C, and G streptococci, is extensively used as a pharmacological agent in thrombolytic therapy and possibly plays a role in streptococcal invasiveness and disease. SK activates Pg to plasmin (Ps) by forming an activator complex with Pg. However, the molecular basis whereby SK binds and activates Pg remains unclear, in part due to the rapid fragmentation of the SK-Pg complex. This study describes a solid phase approach to study this interaction in which a **recombinant** SK molecule was constructed with glutathione-S-transferase appended to the NH2 terminus and (Gly)(3)(His)(8) appended to the COOH terminus. This dual fusion protein molecule, immobilized on either Sepharose-S-hexylglutathione or Ni2+ dinitriloacetic acid-Sepharose was then used to study the interaction of SK with Pg. These SK-Pg complexes exhibited amidolytic and proteolytic activity similar to native SK, but the pattern of fragmentation of the SK molecule was dependent upon whether the SK molecule was immobilized either at its NH2- or COOH terminus. This solid phase approach may contribute to a greater understanding of the role of SK in Pg activation by enabling the 'capture' of intact activator complexes under physiological conditions and, in addition, may serve as a useful model to analyze other bacterial-host protein interactions important in the pathogenesis of disease.

L17 ANSWER 47 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2005:29440 HCAPLUS
DOCUMENT NUMBER: 142:428913
TITLE: An improved process for the simultaneous preparation of extracellular **streptokinase** and its analogues
INVENTOR(S): Dikshit, Kanak Lata; Vyas, Vinay Venkatrao; Mahajan, Ritu; Kaur, Jaodeep; Thapar, Nitika; Phatap, Jitesh; Nihalani, Deepak; Sahni, Girish
PATENT ASSIGNEE(S): Council of Scientific and Industrial Research, India
SOURCE: Indian, 122 pp.
CODEN: INXXAP
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
IN 183828	A	20000429	IN 1994-DE1727	19941230
PRIORITY APPLN. INFO.:			IN 1994-DE1727	19941230

AB Extracellular **streptokinase** and its analogs prepared by growing **recombinant** E.coli in a conventional fermentation medium under

stirring and supplemented with aeration, separating the cells from supernatant by known methods followed by recovering and purifying **Streptokinase** and its analogs from supernatant.

L17 ANSWER 48 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:175570 HCAPLUS
DOCUMENT NUMBER: 132:218864
TITLE: **Streptokinase** analogs with low antigenicity for use as thrombolytics
INVENTOR(S): Torrens Madrazo, Isis Del Carmen; Garcia Ojalvo, Ariana; De La Fuente Garcia, Jose De Jesus; Seralena Menendez, Alina
PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba
SOURCE: Eur. Pat. Appl., 54 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 985729	A2	20000315	EP 1999-202639	19990813
EP 985729	A3	20000531		
EP 985729	B1	20050427		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
AU 9943424	A1	20000309	AU 1999-43424	19990805
AU 769916	B2	20040212		
CA 2277554	C	20041102	CA 1999-2277554	19990806
CA 2277554	AA	20000214		
US 6309873	B1	20011030	US 1999-374038	19990813
AT 294239	E	20050515	AT 1999-202639	19990813
US 6413759	B1	20020702	US 2000-658179	20000908

PRIORITY APPLN. INFO.: CU 1998-119 A 19980814
US 1999-374038 A3 19990813

AB **Streptokinase** analogs with antigenic domains modified to minimize antigenicity are described for use in the treatment of clotting-associated disorders. The proteins retain their capacity for plasminogen activator complex formation. The proteins are manufactured by **expression** of the corresponding allele of the skc2 gene encoding **streptokinase SKC-2** (Heberkinase®). The mols. obtained from present invention can be used in the treatment of disorders as myocardial infarction, pulmonary thromboembolism, surgical complications and other cases of thrombosis.

L17 ANSWER 49 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1999:579938 HCAPLUS
DOCUMENT NUMBER: 131:183948
TITLE: Manufacture of **streptokinase** for therapeutic use by **expression** of the **cloned** gene in Pichia pastoris
INVENTOR(S): Estrada Garcia, Mario; Rubiera Chaplen, Roger; Perez, Hidalgo; Serrano Doce, Ricardo; Hernandez Marrero, Luciano F.; Rodriguez Collazo, Pedro; Castro Ramirez, Anaisel; Munoz Munoz, Emilio Amable; Bravo Martinez, Walfrido; Campos Somavilla, Magalys; Pedraza Fernandez, Alicia; De la Furente Garcia, Jose de J.; Herrera Martinez, Luis S.
PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba
SOURCE: Czech Rep., 18 pp.
CODEN: CZXXED

DOCUMENT TYPE: Patent
LANGUAGE: Czech
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
CZ 284692	B6	19990217	CZ 1991-2256		19910719
RU 2107726	C1	19980327	RU 1991-5001280		19910717
PRIORITY APPLN. INFO.:			CU 1990-90	A	19900523
			CS 1991-2256	A	19910719
			SU 1991-5001280	A	19910717

AB **Streptokinase** for therapeutic use is manufactured by **expression** of the **cloned** gene in Pichia pastoris. The protein may be secreted into the culture medium or accumulated intracellularly. The gene was **cloned** from a type C Streptococcus **equisimilis** by PCR. **Expression** of the gene from the AOX1 promoter using Pichia pastoris as the host resulted in the manufacture of an enzyme with a specific activity of 50,000-100,000 fibrin-agarose units/mg protein.

L17 ANSWER 50 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1999:405093 HCAPLUS
DOCUMENT NUMBER: 131:54027
TITLE: Fibrin-dependent plasminogen activator activity of modified bacterial **streptokinases**
INVENTOR(S): Reed, Guy L.
PATENT ASSIGNEE(S): The President and Fellows of Harvard College, USA
SOURCE: PCT Int. Appl., 73 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 9931247	A1	19990624	WO 1998-US26694		19981215
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM					
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG					
AU 9918295	A1	19990705	AU 1999-18295		19981215
US 6210667	B1	20010403	US 1998-211542		19981215
PRIORITY APPLN. INFO.:			US 1997-69497P	P	19971215
			WO 1998-US26694	W	19981215

AB A pharmaceutical composition in a preferred embodiment comprises an isolated bacterial protein **streptokinase** that induces fibrin-dependent plasminogen activation, and methods for dissolving blood clots in a subject using such a composition Two preferred **streptokinase** mutants and truncated derivs. comprising residues 144-293 and residues 60-414 of the Streptococcus **equisimilis** H46A enzyme. Deletion of the first 59 amino acids to product mutant rSK60-414 yielded a protein with a 767-fold decrease in kcat compared to that of rSK1-414, without any significant change in the Km. The N-terminus dets. the clot (fibrin) dependence of plasminogen activation by **streptokinase** and the regulation of plasminogen activation in the presence of fibrin. By virtue of its requirement for fibrin for plasminogen activation in human plasma, and its sparing of fibrinogen during clot dissoln., **streptokinase** deleted of N-terminal amino acid residues is similar to tissue-type

plasminogen activator. Embodiments also include a nucleic acid encoding such as a bacterial protein, a nucleic acid encoding such a bacterial protein as a fusion to another protein, an **expression** vector with the nucleic acid, and a host cell transformed with the **expression** vector.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 51 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1997:155090 HCAPLUS
DOCUMENT NUMBER: 126:154444
TITLE: **Streptokinases** analogs resistant to cleavage and inactivation by plasmin
INVENTOR(S): Reed, Guy L.
PATENT ASSIGNEE(S): President and Fellows of Harvard College, USA
SOURCE: PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9641883	A1	19961227	WO 1996-US9640	19960607
W: CA, JP, MX				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5854049	A	19981229	US 1995-488940	19950609
PRIORITY APPLN. INFO.:			US 1995-488940 A	19950609

AB **Streptokinase** analogs with altered plasmin-binding features that are resistant to binding by plasmin and subsequent cleavage and inactivation are described for use as thrombolytics with a prolonged serum half-life. Specifically, analogs of the Streptococcus **equisimilis** **streptokinase** are described. Changes that increase plasminogen resistance include alterations of the plasmin-binding domain and and blocking of the N-terminus. Fusion proteins with maltose-binding protein as the N-terminal moiety are prepared and their plasmin resistance and **streptokinase** activity are described. Similarly, analogs with substitutions of basic amino acids that identify internal plasmin and trypsin cleavage sites were prepared and characterized.

L17 ANSWER 52 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1993:229181 HCAPLUS
DOCUMENT NUMBER: 118:229181
TITLE: **Cloning** and **expression** of a gene for **streptokinase** from a hemolytic Streptococcus
INVENTOR(S): Garcia, Mario P. E.; Chaplen, Roger R.; Hidalgo, Aimee P.; Doce, Ricardo S.; Marrero, Luciano F. H.; Collazo, Pedro R.; Ramirez, Anaisel C.; Munoz, Emilio A. M.; Martinez, Walfrido B.; et al.
PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba
SOURCE: Can. Pat. Appl., 26 pp.
CODEN: CPXXEB
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CA 2043953	AA	19921206	CA 1991-2043953	19910605
CA 2043953	C	20010612		

HU 62655	A2	19930528	HU 1991-1770	19910527
HU 216073	B	19990428		
SK 279873	B6	19990507	SK 1991-2256	19910719
PRIORITY APPLN. INFO.:			CA 1991-2043953	19910605

AB The gene for **streptokinase** of a Streptococcus **equisimilis** type C is **cloned** and **expressed** in Escherichia **coli** or in yeasts. **Expression** in yeasts uses the promoter of the AOX1 gene of Pichia pastoris to regulate **expression**. Secretion of the protein was achieved using the cognate signal peptide or one from sucrose invertase. The gene was **cloned** by PCR amplification using different pairs of primers to **clone** the gene with or without the signal sequence. Integrating **expression** vectors for **expression** of the gene in Pichia pastoris with or without secretion of the product were constructed. When the secretory construct was used, **streptokinase** yields of 1-1.2 g/L were obtained. The protein had the expected biol. activities, and the purified enzyme had a specific activity of 50,000-100,000 units/mg.

L17 ANSWER 53 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER:	1992:646505 HCAPLUS
DOCUMENT NUMBER:	117:246505
TITLE:	**Streptokinase** mutation affecting **skc expression** in homologous and heterologous hosts
AUTHOR(S):	Mechold, U.; Muller, J.; Malke, H.
CORPORATE SOURCE:	Cent. Inst. Microbiol. Exp. Ther., Jena, D-6900, Germany
SOURCE:	Zentralblatt fuer Bakteriologie, Supplement (1992), 22(New Perspect. Streptococci Streptococcal Infect.), 336-8 CODEN: ZBASE2; ISSN: 0941-018X
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB Mutations affecting the level of **streptokinase** gene **skc** **expression** and/or secretion in homologous and heterologous hosts are phys. characterized. The principal classes of mutations produced included **skc** deletions, IS element insertions, and **skc** duplications. The deletion events, represented by mutations Δ(**skc**)-247 and Δ(**skc**)-305 present in plasmids pMM247 and pMM305, resp., removed a tetrapeptide (F10-L13 or L12-A15) from the hydrophobic core of the **Skc** signal sequence. These mutations, reduced the size, hydrophobicity and predicted alpha-helicity of the central region of the signal sequence. The corresponding plasmids, upon transformation into E. **coli** and P. mirabilis L-forms, substantially increased the level of **Skc expression** in either host. In E. **coli**, they also facilitated the export of mature **Skc** into the culture medium. In the gram-pos. hosts, **skc expression** was less dramatically affected; however, the proportion of **Skc** activity found in the culture medium was significantly decreased when compared to the extracellular activity resulting from wild type **skc**. IS1 insertion did not alter the primary structure of the promoter but displaced in upward direction, by 768 bp, a static DNA bending locus having its center some 140 bp upstream of the -35 region in wild type DNA. When studied with plasmid pMM697, this insertion event resulted in severely decreased **Skc** **expression** in all hosts but, expectedly, did not affect **Skc** secretability. Gene **skc** duplication in the chromosome of the homologous producer strain, S. **equisimilis** H46A, was achieved by a single crossover event between the chromosomes and an integrateable **Skc** plasmid, pSM752, in the region of shared homol. As judged by Southern hybridization, cells transiently supporting the replication of pSM752 gave rise to a stable erythromycin-resistant **clone** designated H46SM which was plasmid-free and produced **Skc** at levels approx. twice as high as the wild type.

L17 ANSWER 54 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1992:585755 HCAPLUS
DOCUMENT NUMBER: 117:185755
TITLE: High-level **expression** of degraded product-free **streptokinase** in Escherichia **coli** by removal of its putative leader sequence. [Erratum to document cited in CA116(13):122160m]
AUTHOR(S): Park, Seung Kook; Jang, Jeong Su; Kim, Jee Cheon; Chun, Moon Jin; Byun, Si Myung
CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol., Seoul, 130-650, S. Korea
SOURCE: Molecules and Cells (1992), 2(1), 119
CODEN: MOCEEK; ISSN: 1016-8478
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The omission of acknowledgment of a research grant has been corrected The error was not reflected in the abstract or the index entries.

L17 ANSWER 55 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1992:441935 HCAPLUS
DOCUMENT NUMBER: 117:41935
TITLE: **Cloning** and **expression** of **streptokinase** gene of C-type Streptococcus **equisimilis**
PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba
SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
JP 04030794	A2	19920203	JP 1990-201600		19900731
JP 3127298	B2	20010122			
EP 489201	A1	19920610	EP 1990-201930		19900717
EP 489201	B1	19951115			
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE					
AT 130369	E	19951215	AT 1990-201930		19900717
ES 2081909	T3	19960316	ES 1990-201930		19900717
US 5296366	A	19940322	US 1991-703778		19910522
AU 644657	B2	19931216	AU 1991-78101		19910531
RU 2107726	C1	19980327	RU 1991-5001280		19910717
PRIORITY APPLN. INFO.:			CU 1990-90	A	19900523
			SU 1991-5001280	A	19910717

AB The **streptokinase** (I) gene **SKC-2** ,with/without signal sequence, is **cloned** from C-type S. **equisimilis** ATCC-9542 by the polymerase chain reaction method and **expressed** in Escherichia **coli** and yeast for com. manufacture of I. Genomic DNA of the C-type S. **equisimilis** was isolated by the standard method and amplified with primers derived from the nucleotide sequence of **SKC** to get I gene with/without signal sequence. **Expression** of the I gene in E. **coli** and Pichia pastoris MP-36 mutant were shown. The production of I with these microorganisms were ≥350 mg/L and ≥1.2 g/L, resp.

L17 ANSWER 56 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1992:122160 HCAPLUS
DOCUMENT NUMBER: 116:122160
TITLE: High-level **expression** of degraded

product-free **streptokinase** in Escherichia **coli** by removal of its putative leader sequence

AUTHOR(S): Park, Seung Kook; Jang, Jeong Su; Kim, Jee Cheon; Chun, Moon Jin; Byun, Si Myung

CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol., Seoul, 130-650, S. Korea

SOURCE: Molecules and Cells (1991), 1(2), 187-92
CODEN: MOCEEK; ISSN: 1016-8478

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The **expression** vector for **streptokinase** has been constructed from the previously **cloned streptokinase** -coding gene (**skc**) from Streptococcus **equisimilis**. Because of its deleterious effect on the host cell growth, the leader sequence of **skc** was removed and the leader sequence-deleted **skc** was subcloned into the vector pKK223-3, which contains the regulatable tac promoter and rrnB T1T2 transcription terminator, with a short synthetic oligonucleotide adapter. When this vector, pKS601 having **skc** gene, was **expressed** in E. **coli**, a 47.4-kDa protein was found to be newly accumulated to about 12% of the total cellular proteins, and it was identified as the **streptokinase** by immunoblotting with rabbit anti-**streptokinase** polyclonal serum. The **expressed streptokinase** was free from carboxyl-terminal degraded 44-kDa **streptokinase** and purified to near homogeneity using DEAE-cellulose and Sephadex G-150 columns. Its specific activity was about 1.3 + 105 CLN units/mg protein.

L17 ANSWER 57 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:672660 HCAPLUS

DOCUMENT NUMBER: 115:272660

TITLE: **Recombinant** of thrombolytic and fibrinolytic enzymes as inactive dimers linked by sequence recognized by blood coagluation factors

INVENTOR(S): Dawson, Keith Martyn; Hunter, Michael George; Czaplewski, Lloyd George

PATENT ASSIGNEE(S): British Bio-Technology Ltd., UK

SOURCE: PCT Int. Appl., 110 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 4

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9109125	A1	19910627	WO 1990-GB1911	19901207
W: AU, CA, FI, HU, JP, KR, NO, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
CA 2069085	AA	19910608	CA 1990-2069085	19901207
CA 2069085	C	20000201		
CA 2069105	AA	19910608	CA 1990-2069105	19901207
AU 9169540	A1	19910718	AU 1991-69540	19901207
AU 644399	B2	19931209		
ZA 9009853	A	19920826	ZA 1990-9853	19901207
ZA 9009854	A	19920826	ZA 1990-9854	19901207
EP 504241	A1	19920923	EP 1991-900869	19901207
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 155812	E	19970815	AT 1991-900851	19901207
ES 2106073	T3	19971101	ES 1991-900851	19901207
IL 96601	A1	19990509	IL 1990-96601	19901207
JP 05502374	T2	19930428	JP 1991-501314	19911115
JP 2900606	B2	19990602		
US 5434073	A	19950718	US 1992-854596	19920603

FI 9202609	A	19920605	FI 1992-2609		19920605
NO 9202237	A	19920806	NO 1992-2237		19920605
AU 9344976	A1	19931118	AU 1993-44976		19930830
PRIORITY APPLN. INFO.:			GB 1989-27722	A	19891207
			WO 1990-GB1911	A	19901207

AB Fibrinolytic or thrombolytic enzymes are manufactured in a **recombinant** host as inactive fusion proteins containing two or more sequences of the protein linked by a peptide that can be cleaved by a blood-coagulation factor. The construction of **expression** vectors for the manufacture of hirudin or **streptokinase** dimers linked by peptides cleavable by Factor Xa or thrombin for Escherichia col or Saccharomyces cerevisiae (with or without product secretion) is described. All the products tested were cleavable by the appropriate factors.

L17 ANSWER 58 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:443480 HCAPLUS
DOCUMENT NUMBER: 115:43480
TITLE: Synthetic genes for **streptokinase** and **streptokinase** analogs and their **expression** in Escherichia **coli**
INVENTOR(S): Fujii, Setsuro; Katano, Tamiki; Majima, Eiji; Ogino, Koichi; Ono, Kenji; Sakata, Yasuyo; Uenoyama, Tsutomu
PATENT ASSIGNEE(S): Otsuka Pharmaceutical Factory, Inc., Japan
SOURCE: Eur. Pat. Appl., 76 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
EP 407942	A2	19910116	EP 1990-113099		19900709
EP 407942	A3	19910904			
EP 407942	B1	19951011			
R: AT, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE					
JP 04011892	A2	19920116	JP 1990-179851		19900706
US 5240845	A	19930831	US 1990-549049		19900706
AU 9058806	A1	19910117	AU 1990-58806		19900709
AU 648029	B2	19940414			
AT 129014	E	19951015	AT 1990-113099		19900709
ES 2078925	T3	19960101	ES 1990-113099		19900709
CA 2020828	AA	19910112	CA 1990-2020828		19900710
PRIORITY APPLN. INFO.:			JP 1989-179432	A	19890711
			JP 1989-307957	A	19891127
			JP 1990-96830	A	19900411

AB Genes encoding **streptokinase** (I) and its derivs. are synthesized and **expressed** in a host such as Escherichia **coli** for manufacture of I suitable for clin. application. The DNA encoding natural-type I was synthesized by standard chemical and used for construction of **expression** plasmid pSKXT, which in turn **expressed** the I gene using the E. **coli** tac promoter and the blc signal sequence. Efficient **expression** of the gene in the E. **coli** transformants and purification of the protein product were demonstrated. I analogs with a carboxy-terminal deletions, optionally with internal modifications were also described.

L17 ANSWER 59 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:179754 HCAPLUS
DOCUMENT NUMBER: 114:179754
TITLE: Fusion proteins of **streptokinase** and human plasminogen
INVENTOR(S): Malke, Horst; Ferretti, Joseph J.
PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Zentralinstitut

fuer Mikrobiologie und Experimentelle Therapie, Ger. Dem. Rep.
SOURCE: Ger. (East), 42 pp.
CODEN: GEXXA8
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 284484	A5	19901114	DD 1989-328631	19890516

PRIORITY APPLN. INFO.: DD 1989-328631 19890516

AB A hybrid **streptokinase** is produced in prokaryotic cells. The **streptokinase**, which displays thrombin selectivity, consists of the N-terminal kringle domains of human plasminogen fused to C-terminal Streptococcus **equisimilis streptokinase**. Escherichia **coli** transformed with plasmids encoding the described fusion protein fused to the N-terminal hexapeptide of β-galactosidase produced the hybrid **streptokinase** which was purified from cell lysates by immuno-affinity chromatog. and by chromatog. on lysine Sepharose.

L17 ANSWER 60 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:162447 HCAPLUS
DOCUMENT NUMBER: 114:162447
TITLE: **Recombinant** manufacture of **streptokinase**
INVENTOR(S): Laplace, Frank; Mueller, Joerg; Malke, Horst
PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Patentabteilung, Ger. Dem. Rep.
SOURCE: Ger. (East), 9 pp.
CODEN: GEXXA8
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 282709	A5	19900919	DD 1988-323948	19881227

PRIORITY APPLN. INFO.: DD 1988-323948 19881227

AB **Streptokinase** from Streptococcus **equisimilis** serotype C is manufactured by **expression** of the **skc** gene in Escherichia **coli**, Bacillus subtilis, or other Streptococcus. The natural **expression** cassette for the **skc** gene was introduced into a broad host-range vector to give plasmid pMLS10. Transformants of Streptococcus sanguis carrying this vector produced 750-1,000 **streptokinase** units/mL in a complex medium after 16 h growth at 36°, at this point the culture reached stationary phase and the enzyme continued to be slowly accumulated.

L17 ANSWER 61 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:96194 HCAPLUS
DOCUMENT NUMBER: 114:96194
TITLE: The leader sequence of **streptokinase** is responsible for its post-translational carboxyl-terminal cleavage
AUTHOR(S): Park, Seung Kook; Lee, Byeong Ryong; Byun, Si Myung
CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol., Seoul, 130-650, S. Korea
SOURCE: Biochemical and Biophysical Research Communications (1991), 174(1), 282-6
CODEN: BBRCA9; ISSN: 0006-291X

DOCUMENT TYPE: Journal
LANGUAGE: English
AB When the **streptokinase** gene from Streptococcus equisimillis was **expressed** from 2 tac promoter-controlled **expression** vectors, one deleted the putative leader sequence of **streptokinase** . Both normal and degraded **streptokinase** were detected in proteins **expressed** from the leader-encoding vector, but only normal **streptokinase** was detected from the leader-deleted vector. These findings indicate that the characteristic carboxyl-terminal cleavage of **streptokinase** is correlated with its leader sequence and occurs during defective secretion. A homogeneous preparation of **streptokinase** was facilitated by **expression** from this leader-deleted vector.

L17 ANSWER 62 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1989:206803 HCAPLUS
DOCUMENT NUMBER: 110:206803
TITLE: **Streptokinase** mutations relieving Escherichia **coli** K-12 (prlA4) of detriments caused by the wild-type **skc** gene
AUTHOR(S): Mueller, Joerg; Reinert, Hilmer; Malke, Horst
CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Therapy, Acad. Sci. G. D. R., Jena, 6900, Ger. Dem. Rep.
SOURCE: Journal of Bacteriology (1989), 171(4), 2202-8
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A novel phenotype is described for E. **coli** K-12 carrying the prlA4 allele determining a membrane component of the protein export mechanism. It is manifest as transformation deficiency for plasmids containing the **cloned** group C streptococcal **streptokinase** gene, **skc**. **Streptokinase** plasmid mutations relieving the prlA4 strain of this deficiency fell into three classes. Class 1 included **skc**::IS5 insertions, with IS5 integrated in a region encoding the **Skc** signal sequence and inactivating **skc**. Class 2 included IS1 insertions leaving **skc** intact but reducing **skc expression**, presumably by altering the function of the **skc** promoter as judged by an insertion site close to the -35 region. Class 3 included **skc** deletions removing the entire signal sequence or a tetrapeptide from its hydrophobic core. The tetrapeptide deletion reduced the size, hydrophobicity, and predicted α-helicity of the central region of the **Skc** signal sequence but facilitated the export of mature **Skc** in both the wild type and the prlA4 mutant. These findings indicate that the incompatibility between prlA4 and **skc** is related to deleterious effects of the **Skc** signal sequence. The tetrapeptide deletion may function by altering the conformation of the signal sequence so as to render interaction with both the PrlA wild-type protein and the PrlA4 mutant protein less detrimental to the export mechanism. These findings also provide an explanation for the difficulties encountered in **cloning streptokinase** genes in E. **coli** plasmids and maintaining their structural stability.

L17 ANSWER 63 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1988:487410 HCAPLUS
DOCUMENT NUMBER: 109:87410
TITLE: Methylotrophic yeast as vehicles for heterologous gene **expression**
AUTHOR(S): Stroman, D. W.; Hagenson, M. J.
CORPORATE SOURCE: Phillips Res. Cent., Phillips Pet. Co., OK, USA
SOURCE: DECHEMA Monographien (1987), 105(Physiol. Genet. Modulation Prod. Form.), 141-6
CODEN: DMDGAG; ISSN: 0070-315X
DOCUMENT TYPE: Journal

LANGUAGE: English
AB The methylotrophic yeast, Pichia pastoris, has been developed as a superior **recombinant** DNA (rDNA) production host. The key component in the development of this host was the **cloning** of the alc. oxidase gene and use of its promoter-regulatory region to control gene **expression**. Heterologous **expression** of several foreign genes in this yeast has been studied. The promoter-regulatory region from the alc. oxidase gene permits very high per cell levels of gene **expression** in an easily regulated manner. These high per cell levels of **expression** can be combined with high cell d. fermentation technol. to yield very high per L production of rDNA products. This is shown by the high levels of production of **streptokinase** in this yeast.

L17 ANSWER 64 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1987:472094 HCAPLUS
DOCUMENT NUMBER: 107:72094
TITLE: New **cloning** vectors for Escherichia **coli** and Bacillus subtilis
INVENTOR(S): Klessen, Christian; Malke, Horst
PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Ger. Dem. Rep.
SOURCE: Ger. (East), 7 pp.
CODEN: GEXXA8
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 231083	A1	19851218	DD 1984-264255	19840619
PRIORITY APPLN. INFO.:			DD 1984-264255	19840619

AB New **cloning** vectors for Escherichia **coli** and Bacillus subtilis, derived from the bifunctional plasmid pGR71 by insertion of a promoter-containing DNA fragment into the unique HindIII site of pGR71 upstream of the chloramphenicol acetyltransferase gene, are described. The new plasmids pSM1711, pSM7711, and pSM7712 contain a Streptococcus promoter from plasmids pMF1, pSM10, or pSM7.

L17 ANSWER 65 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1986:509637 HCAPLUS
DOCUMENT NUMBER: 105:109637
TITLE: The **streptokinase** gene
AUTHOR(S): Malke, H.; Ferretti, J. J.
CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Ther., Ger. Acad. Sci., Jena, DDR-6900, Ger. Dem. Rep.
SOURCE: Folia Haematologica (Leipzig) (1986), 113(1-2), 88-98
CODEN: FOHEAW; ISSN: 0323-4347
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Streptococcus **equisimilis** Gene **skc** for **streptokinase** [9002-01-1] was **cloned** on the vector L47 and then subcloned into plasmids pACYC184 and pBR322 to form **recombinant** plasmids pMF2 (10.4 kb) and pMF5 (9.9 kb), resp., for **expression** in Escherichia **coli**. Plasmid pMF5 contained a 2568-base-pair (bp) insert that included the 1320-bp coding sequence for prestreptokinase. The prestreptokinase comprised 440 amino acid residues, including a 26-amino acid signal peptide. The insert also contained the **skc** upstream regions involved in the regulation of transcription and translation and a 15-bp repeat located 34 bp downstream of the **skc** translation stop signal, which very likely represents the rho-independent transcription terminator. The **skc** gene showed no extended regions homologous to the staphylokinase gene. Heterologous **skc** gene **expression** was also attained in S. sanguis after subcloning of the gene of pMF5 onto plasmid pSM7 to form the

bifunctional shuttle plasmid pSM752 (13.3 kb). Plasmid pSM752 was not only functional in E. **coli** and S. sanguis, but also in Bacillus subtilis. The **cloned streptokinase expressed** in E. **coli**, S. sanguis, or B. subtilis has the same specificity as that of the donor strain.

L17 ANSWER 66 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1986:473709 HCAPLUS
DOCUMENT NUMBER: 105:73709
TITLE: **Cloning** of streptococcal genes with Streptococcus-Escherichia **coli** shuttle vector pSA3
AUTHOR(S): Dao, M. L.; Ferretti, J. J.
CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, USA
SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis., 9th (1985), Meeting Date 1984, 233-4. Editor(s): Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi. Reedbooks: Bracknell, UK.
CODEN: 55BSAN
DOCUMENT TYPE: Conference
LANGUAGE: English
AB A shuttle vector, the chimeric plasmid pSA3, which can replicate in both E. **coli** and S. sanguis, was constructed. Chromosomal DNA from S. mutans was ligated into this plasmid and **cloned** in E. **coli**. Of 472 **clones** tested, 43 **clones expressed** S. mutans surface antigens. A **cloned** S. **equisimilis streptokinase** [9002-01-1] gene was inserted into plasmid pSA3 and then used to transform E. **coli**, S. sanguis, and S. mutans, all of which **expressed** the **cloned streptokinase** gene.

L17 ANSWER 67 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1986:473707 HCAPLUS
DOCUMENT NUMBER: 105:73707
TITLE: **Cloned streptokinase** gene from Streptococcus **equisimilis** H46A
AUTHOR(S): Malke, H.; Ferretti, J. J.
CORPORATE SOURCE: Ger. Acad. Sci., Jena, Ger. Dem. Rep.
SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis., 9th (1985), Meeting Date 1984, 221-2. Editor(s): Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi. Reedbooks: Bracknell, UK.
CODEN: 55BSAN
DOCUMENT TYPE: Conference
LANGUAGE: English
AB The **streptokinase** [9002-01-1] gene **skc** of S. **equisimilis** was **cloned** in Escherichia **coli** with plasmid pBR322. **Expression** of gene **skc** was observed with both orientations of the gene, which indicated that its own promoter was present and was functional in E. **coli**. **Streptokinase** was excreted by the E. **coli** host. The gene contained a 1320-base-pair open reading frame which encodes 440 amino acids, including a signal peptide of 26 amino acids.

L17 ANSWER 68 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1986:455636 HCAPLUS
DOCUMENT NUMBER: 105:55636
TITLE: The **streptokinase** gene: **cloning**, sequencing and **expression** in new hosts
AUTHOR(S): Malke, Horst
CORPORATE SOURCE: Zentralinst. Mikrobiol., Dtsch. Akad. Wiss., Jena,

Ger. Dem. Rep.
SOURCE: Zeitschrift fuer Klinische Medizin (1985) (1986), 41(7), 502-4
CODEN: ZKMEEF; ISSN: 0233-1608
DOCUMENT TYPE: Journal
LANGUAGE: German

AB The **streptokinase** (I) [9002-01-1] gene (**skc**) of Streptococcus **equisimilis** H46A was **cloned** in Escherichia **coli** using vector λL47. One of the **recombinant clones** was used to subclone **skc** in E. **coli** plasmid vectors. Plasmids pMF2 (10.4 kilobases, composed of pACYC184 plus a 6.4-kilobase EcoRI fragment) and pMF5 (6.9 kilobases, with a 2.5-kilobase fragment in the PstI site of pBR322) determined I formation in E. **coli**; **expression** of **skc** was independent of its orientation, indicating that the complete gene, together with its control elements, was present. The 2.5-kilobase PstI fragment of pMF5 was isolated and sequenced in the M13 system. Of 2568 base pairs, the largest open reading frame consisted of 1320 base pairs coding for prestreptokinase, corresponding to I plus its 26-amino acid leader sequence. **Expression** of **skc** was attained in S. sanguis after transformation with the shuttle vector pSM752. In fermentation expts., I production rates of 1500 U/mL were attained, which was below the levels obtained with S. **equisimilis**. Use of pSM752 for similar transformation of Bacillus subtilis is briefly discussed.

L17 ANSWER 69 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1985:608113 HCAPLUS
DOCUMENT NUMBER: 103:208113
TITLE: **Streptokinase**-coding **recombinant** vectors
INVENTOR(S): Ferretti, Joseph J.; Malke, Horst
PATENT ASSIGNEE(S): Phillips Petroleum Co. , USA
SOURCE: Eur. Pat. Appl., 21 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 151337	A2	19850814	EP 1984-306851	19841008
EP 151337	A3	19861008		
R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE				
DD 249037	A1	19870826	DD 1983-255523	19831010
US 4764469	A	19880816	US 1984-585417	19840302
AU 8433859	A1	19850418	AU 1984-33859	19841005
AU 561372	B2	19870507		
ZA 8407873	A	19850529	ZA 1984-7873	19841008
AT 61816	E	19910415	AT 1984-306851	19841008
FI 8403963	A	19850411	FI 1984-3963	19841009
NO 8404039	A	19850411	NO 1984-4039	19841009
DK 8404822	A	19850426	DK 1984-4822	19841009
JP 60237995	A2	19851126	JP 1984-212403	19841009
ES 536623	A1	19870116	ES 1984-536623	19841009
CA 1223223	A1	19870623	CA 1984-464939	19841009
DD 273284	A5	19891108	DD 1984-268254	19841010
US 5066589	A	19911119	US 1988-212254	19880627
US 5187098	A	19930216	US 1992-888420	19920522

PRIORITY APPLN. INFO.:

DD 1983-255523	A	19831010
US 1984-585417	A	19840302
EP 1984-306851	A	19841008
US 1988-212254	A2	19880627
US 1989-348206	B1	19890509

AB **Recombinant** vectors that code for **streptokinase** [9002-01-1] are constructed and **cloned** in Escherichia **coli**. Thus, DNA from Streptococcus **equisimilis** was isolated and digested with the restriction endonuclease Sau3A. DNA fragments of between 4-15 kb were **cloned** into phage λ L47. The ligated phage was infectively added to E. **coli** lawns and **streptokinase**-producing **clones** were isolated. The DNA from one such **clone**, λ L47E **skc** was partially digested with HindIII and then inserted into the HindIII site of plasmid pBR322. The **recombinant** plasmids were used to transform E. **coli** strain HB101. The plasmid isolated from 1 **streptokinase**-producing strain, pMF1, was isolated and a restriction map was prepared A nucleotide sequence anal. of pMF1 showed that the **cloned** fragment encoded for **streptokinase** as well as an amino-terminal signal peptide which is bound to **streptokinase** and which is hydrolyzed during a **streptokinase** secretion event.

L17 ANSWER 70 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1985:107414 HCAPLUS
DOCUMENT NUMBER: 102:107414
TITLE: Streptococcus-Escherichia **coli** shuttle vector pSA3 and its use in the **cloning** of streptococcal genes
AUTHOR(S): Dao, My Lien; Ferretti, Joseph J.
CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, 73190, USA
SOURCE: Applied and Environmental Microbiology (1985), 49(1), 115-19
CODEN: AEMIDF; ISSN: 0099-2240
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A shuttle vector that can replicate in both Streptococcus and E. **coli** was constructed by joining the E. **coli** plasmid pACYC184 (chloramphenicol [56-75-7] and tetracycline [60-54-8] resistance) to the streptococcal plasmid pGB305 (erythromycin [114-07-8] resistance). The resulting chimeric plasmid is designated pSA3 (chloramphenicol, erythromycin, and tetracycline resistance) and had 7 unique restriction sites: EcoRI, EcoRV, BamHI, SalI, XbaI, NruI, and SphI. Mol. **cloning** into the EcoRI or EcoRV site results in inactivation of chloramphenicol resistance, and **cloning** into the BamHI, SalI site results in inactivation of tetracycline resistance in E. **coli**. Plasmid pSA3 was transformed and was stable in S. sanguis and S. mutans in the presence of erythromycin. Plasmid pSA3 was used to construct a library of the S. mutans GS5 genome in E. **coli**, and **expression** of surface antigens in this heterologous host was confirmed with S. mutans antiserum. A previously **cloned** determinant that species **streptokinase** [9002-01-1] was subcloned into pSA3, and this **recombinant** plasmid was stable in the presence of a selective pressure and **expressed** **streptokinase** activity in E. **coli**, S. sanguis, and S. mutans.

L17 ANSWER 71 OF 71 LIFESCI COPYRIGHT 2005 CSA on STN
ACCESSION NUMBER: 96:44932 LIFESCI
TITLE: Functional analysis of a relA/spoT gene homolog from Streptococcus **equisimilis**
AUTHOR: Mechold, U.; Cashel, M.; Steiner, K.; Gentry, D.; Malke, H.
CORPORATE SOURCE: Inst. Molecular Biol., Jena Univ., Winzerlaer Str. 10, D-07745 Jena, Germany
SOURCE: J. BACTERIOL., (1996) vol. 178, no. 5, pp. 1404-1411. ISSN: 0021-9193.
DOCUMENT TYPE: Journal
FILE SEGMENT: J; G

LANGUAGE: English
SUMMARY LANGUAGE: English
AB We examined the functional attributes of a gene encountered by sequencing the **streptokinase** gene region of Streptococcus **equisimilis** H46A. This gene, originally called rel, here termed rel sub( )S. **equisimilis**, is homologous to two related Escherichia **coli** genes, spoT and relA, that function in the metabolism of guanosine 5',3'-polyphosphates [(p)ppGpp]. Studies with a variety of E. **coli** mutants led us to deduce that the highly **expressed** rel sub( )S. **equisimilis** gene encodes a strong (p)ppGppase and a weaker (p)ppGpp synthetic activity, much like the spoT gene, with a net effect favoring degradation and no complementation of the absence of the relA gene. We verified that the Rel sub( )S. **equisimilis** protein, purified from an E. **coli** relA spoT double mutant, catalyzed a manganese-activated (p)ppGpp 3'-pyrophosphohydrolase reaction similar to that of the SpoT enzyme. This Rel sub( )S. **equisimilis** protein preparation also weakly catalyzed a ribosome-independent synthesis of (p)ppGpp by an ATP to GTP 3'-pyrophosphoryltransferase reaction when degradation was restricted by the absence of manganese ions. An analogous activity has been deduced for the SpoT protein from genetic evidence. In addition, the Rel sub( )S. **equisimilis** protein displays immunological cross-reactivity with polyclonal antibodies specific for SpoT but not for RelA. Despite assignment of rel sub( )S. **equisimilis** gene function in E. **coli** as being similar to that of the native spoT gene, disruptions of rel sub( )S. **equisimilis** in S. **equisimilis** abolish the parental (p)ppGpp accumulation response to amino acid starvation in a manner expected for relA mutants rather than spoT mutants.

=> d his

(FILE 'HOME' ENTERED AT 17:11:46 ON 29 DEC 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005

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L1        42118 S STREPTOKINASE?
L2          630 S "SKC" OR "SKC-2"
L3        42539 S L1 OR L2
L4      7454183 S CLON? OR EXPRESS? OR RECOMBINANT
L5         4154 S L3 AND L4
L6         1362 S EQUISIMILIS
L7          220 S L5 AND L6
L8        49433 S INCLUSION (W) BOD?
L9            5 S L7 AND L8
L10           1 DUP REM L9 (4 DUPLICATES REMOVED)
L11         106 DUP REM L7 (114 DUPLICATES REMOVED)
L12           5 S LAMNDA
L13           1 S L11 AND INCLUSION
L14           0 S L11 AND AGGREGAT?
L15           0 S L11 AND INSOLUBLE
L16           1 S L11 AND SOLUBL?
L17          71 S L11 AND COLI
```

=> e kuppusamy m/au

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E1            1     KUPPUSAMY KAVITHA/AU
E2            3     KUPPUSAMY KAVITHA T/AU
E3           40 --> KUPPUSAMY M/AU
E4            2     KUPPUSAMY M R/AU
E5            1     KUPPUSAMY MUSAVAN/AU
E6            2     KUPPUSAMY N/AU
E7            2     KUPPUSAMY NALLAGOUNDER/AU
E8          494     KUPPUSAMY P/AU
E9            1     KUPPUSAMY PARIANNAN/AU
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E10           1     KUPPUSAMY PERIANNAM/AU
E11         297     KUPPUSAMY PERIANNAN/AU
E12           1     KUPPUSAMY R/AU

=> s e3
L18          40 "KUPPUSAMY M"/AU

=> e ella k/au
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E2            7     ELLA JUHA/AU
E3            0 --> ELLA K/AU
E4            1     ELLA K A/AU
E5           53     ELLA K M/AU
E6            1     ELLA KAZACHKOVA/AU
E7            1     ELLA KHALID A ABOU/AU
E8            1     ELLA KIRSHNA M/AU
E9            1     ELLA KRISHNA E/AU
E10          28     ELLA KRISHNA M/AU
E11           2     ELLA KRISHNA MURTHY/AU
E12           1     ELLA KUSHNIR/AU

=> e khatri g s/au
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E2           33     KHATRI G R/AU
E3           46 --> KHATRI G S/AU
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E5            1     KHATRI GAJENDRA K/AU
E6            1     KHATRI GAURAV/AU
E7           15     KHATRI GHAN SHYAM/AU
E8            2     KHATRI GOPAL KRISHAN/AU
E9            1     KHATRI GS/AU
E10           1     KHATRI GULSHAN R/AU
E11           3     KHATRI H/AU
E12           1     KHATRI H K/AU

=> s e3
L19          46 "KHATRI G S"/AU

=> e lahiri s/au
E1            6     LAHIRI RANGAN/AU
E2            4     LAHIRI ROMA/AU
E3         1660 --> LAHIRI S/AU
E4            2     LAHIRI S */AU
E5            8     LAHIRI S A/AU
E6           16     LAHIRI S B/AU
E7          610     LAHIRI S C/AU
E8            1     LAHIRI S C */AU
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E10          18     LAHIRI S D/AU
E11           1     LAHIRI S H/AU
E12         276     LAHIRI S K/AU

=> s e3
L20        1660 "LAHIRI S"/AU

=> e srinivas v k/au
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E2            1     SRINIVAS V B S/AU
E3           28 --> SRINIVAS V K/AU
E4            1     SRINIVAS V N S/AU
E5           89     SRINIVAS V R/AU
E6           43     SRINIVAS V S/AU
E7            1     SRINIVAS V SEENA/AU
E8            1     SRINIVAS V SESHA/AU
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E9            1     SRINIVAS V T/AU
E10           7     SRINIVAS V V/AU
E11           2     SRINIVAS VANKEEPURAM S/AU
E12           2     SRINIVAS VELLIMEDU KANNAPPA/AU

=> s e3
L21          28 "SRINIVAS V K"/AU

=> d his

     (FILE 'HOME' ENTERED AT 17:11:46 ON 29 DEC 2005)

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L1         42118 S STREPTOKINASE?
L2           630 S "SKC" OR "SKC-2"
L3         42539 S L1 OR L2
L4       7454183 S CLON? OR EXPRESS? OR RECOMBINANT
L5          4154 S L3 AND L4
L6          1362 S EQUISIMILIS
L7           220 S L5 AND L6
L8         49433 S INCLUSION (W) BOD?
L9             5 S L7 AND L8
L10            1 DUP REM L9 (4 DUPLICATES REMOVED)
L11          106 DUP REM L7 (114 DUPLICATES REMOVED)
L12            5 S LAMNDA
L13            1 S L11 AND INCLUSION
L14            0 S L11 AND AGGREGAT?
L15            0 S L11 AND INSOLUBLE
L16            1 S L11 AND SOLUBL?
L17           71 S L11 AND COLI
                 E KUPPUSAMY M/AU
L18           40 S E3
                 E ELLA K/AU
                 E KHATRI G S/AU
L19           46 S E3
                 E LAHIRI S/AU
L20         1660 S E3
                 E SRINIVAS V K/AU
L21           28 S E3

=> s l17 or l18 or l19 or l20 or l21
L22        1842 L17 OR L18 OR L19 OR L20 OR L21

=> s l3 and l22
L23          71 L3 AND L22

=> s l8 and l23
L24           1 L8 AND L23

=> d all

L24  ANSWER 1 OF 1  BIOSIS  COPYRIGHT (c) 2005 The Thomson Corporation  on STN
AN   2000:96119  BIOSIS
DN   PREV200000096119
TI   Two streptokinase genes are expressed with different
     solubility in Escherichia coli W3110.
AU   Pupo, Elder [Reprint author]; Baghbaderani, Behnam A.; Lugo, Victoria;
     Fernandez, Julio; Paez, Rolando; Torrens, Isis
CS   Biopharmaceutical Development Division, Center for Genetic Engineering and
     Biotechnology, Havana, Cuba
SO   Biotechnology Letters, (Dec., 1999) Vol. 21, No. 12, pp. 1119-1123. print.
     CODEN: BILED3. ISSN: 0141-5492.
DT   Article
```

LA English
ED Entered STN: 15 Mar 2000
Last Updated on STN: 3 Jan 2002
AB The **streptokinase** (SK) gene from S. **equisimilis** H46A (ATCC 12449) was **cloned** in E. **coli** W3110 under the control of the tryptophan promoter. The **recombinant** SK, which represented 15% of total cell protein content, was found in the soluble fraction of disrupted cells. The solubility of this SK notably differed from that of the product of the SK gene from S. **equisimilis** (ATCC 9542) which had been **cloned** in E. **coli** W3110 by using similar **expression** vector and cell growth conditions, and occurred in the form of **inclusion bodies**.
CC Genetics of bacteria and viruses 31500
Biochemistry methods - Nucleic acids, purines and pyrimidines 10052
Biochemistry methods - Proteins, peptides and amino acids 10054
Replication, transcription, translation 10300
Biophysics - Molecular properties and macromolecules 10506
Microbiological apparatus, methods and media 32000
Food microbiology - General and miscellaneous 39008
Enzymes - General and comparative studies: coenzymes 10802
Metabolism - Proteins, peptides and amino acids 13012
Morphology and cytology of bacteria 30500
Physiology and biochemistry of bacteria 31000
IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Molecular Genetics (Biochemistry and Molecular Biophysics)
IT Chemicals & Biochemicals
amino acids; enzymes; proteins; tryptophan
IT Miscellaneous Descriptors
biotechnology; cell growth conditions; **expression** vectors; gene **expression**; promoters; tryptophan promoter
ORGN Classifier
Enterobacteriaceae 06702
Super Taxa
Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria; Microorganisms
Organism Name
Escherichia **coli**: W 3110
Taxa Notes
Bacteria, Eubacteria, Microorganisms
ORGN Classifier
Gram-Positive Cocci 07700
Super Taxa
Eubacteria; Bacteria; Microorganisms
Organism Name
Streptococcus **equisimilis**
Taxa Notes
Bacteria, Eubacteria, Microorganisms
RN 54-12-6Q (tryptophan)
73-22-3Q (tryptophan)

=> d his

(FILE 'HOME' ENTERED AT 17:11:46 ON 29 DEC 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005
L1 42118 S STREPTOKINASE?
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L5 4154 S L3 AND L4
L6 1362 S EQUISIMILIS

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L7          220 S L5 AND L6
L8        49433 S INCLUSION (W) BOD?
L9            5 S L7 AND L8
L10           1 DUP REM L9 (4 DUPLICATES REMOVED)
L11         106 DUP REM L7 (114 DUPLICATES REMOVED)
L12           5 S LAMNDA
L13           1 S L11 AND INCLUSION
L14           0 S L11 AND AGGREGAT?
L15           0 S L11 AND INSOLUBLE
L16           1 S L11 AND SOLUBL?
L17          71 S L11 AND COLI
                E KUPPUSAMY M/AU
L18          40 S E3
                E ELLA K/AU
                E KHATRI G S/AU
L19          46 S E3
                E LAHIRI S/AU
L20        1660 S E3
                E SRINIVAS V K/AU
L21          28 S E3
L22        1842 S L17 OR L18 OR L19 OR L20 OR L21
L23          71 S L3 AND L22
L24           1 S L8 AND L23

=> s l4 and l23
L25          71 L4 AND L23

=> s l6 and l25
L26          71 L6 AND L25

=> d his

     (FILE 'HOME' ENTERED AT 17:11:46 ON 29 DEC 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005
L1         42118 S STREPTOKINASE?
L2           630 S "SKC" OR "SKC-2"
L3         42539 S L1 OR L2
L4       7454183 S CLON? OR EXPRESS? OR RECOMBINANT
L5          4154 S L3 AND L4
L6          1362 S EQUISIMILIS
L7           220 S L5 AND L6
L8         49433 S INCLUSION (W) BOD?
L9             5 S L7 AND L8
L10            1 DUP REM L9 (4 DUPLICATES REMOVED)
L11          106 DUP REM L7 (114 DUPLICATES REMOVED)
L12            5 S LAMNDA
L13            1 S L11 AND INCLUSION
L14            0 S L11 AND AGGREGAT?
L15            0 S L11 AND INSOLUBLE
L16            1 S L11 AND SOLUBL?
L17           71 S L11 AND COLI
                 E KUPPUSAMY M/AU
L18           40 S E3
                 E ELLA K/AU
                 E KHATRI G S/AU
L19           46 S E3
                 E LAHIRI S/AU
L20         1660 S E3
                 E SRINIVAS V K/AU
L21           28 S E3
L22         1842 S L17 OR L18 OR L19 OR L20 OR L21
L23           71 S L3 AND L22
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L24 1 S L8 AND L23
L25 71 S L4 AND L23
L26 71 S L6 AND L25

=> d 1-71 ibib ab

L26 ANSWER 1 OF 71 MEDLINE on STN
ACCESSION NUMBER: 2002322701 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12065504
TITLE: Dual control of **streptokinase** and streptolysin S production by the covRS and fasCAX two-component regulators in Streptococcus dysgalactiae subsp. **equisimilis**.
AUTHOR: Steiner Kerstin; Malke Horst
CORPORATE SOURCE: Institute for Molecular Biology, Friedrich Schiller University Jena, D-07745 Jena, Germany.
SOURCE: Infection and immunity, (2002 Jul) 70 (7) 3627-36.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AY075106; GENBANK-AY075107
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020615
Last Updated on STN: 20020731
Entered Medline: 20020730

AB Synthesis of the plasminogen activator **streptokinase** (SK) by group A streptococci (GAS) has recently been shown to be subject to control by two two-component regulators, covRS (or csrRS) and fasBCA. In independent studies, response regulator CovR proved to act as the repressor, whereas FasA was found to act indirectly as the activator by controlling the **expression** of a stimulatory RNA, fasX. In an attempt at understanding the regulation of SK production in the human group C streptococcal (GCS) strain H46A, the strongest SK producer known yet, we provide here physical and functional evidence for the presence of the cov and fas systems in GCS as well and, using a mutational approach, compare the balance between their opposing actions in H46A and GAS strain NZ131. Sequence analysis combined with Southern hybridization revealed that the covRS and fasCAX operons are preserved at high levels of primary structure identity between the corresponding GAS and GCS genes, with the exception of fasB, encoding a second sensor kinase that is not a member of the GCS fas operon. This analysis also showed that wild-type H46A is actually a derepressed mutant for SK and streptolysin S (SLS) synthesis, carrying a K102 amber mutation in covR. Using cov and fas mutations in various combinations together with strain constructs allowing complementation in trans, we found that, in H46A, cov and fas contribute to approximately equal negative and positive extents, respectively, to constitutive SK and SLS activity. The amounts of SK paralleled the level of **skc**(H46A) transcription. The most profound difference between H46A and NZ131 regarding the relative activities of the cov and fas systems consisted in significantly higher activity of a functional CovR repressor in NZ131 than in H46A. In NZ131, CovR decreased SK activity in a Fas(+) background about sevenfold, compared to a 1.9-fold reduction of SK activity in H46A. Combined with the very short-lived nature of covR mRNA (decay rate, 1.39/min), such differences may contribute to strain-specific peculiarities of the **expression** of two prominent streptococcal virulence factors in response to environmental changes.

L26 ANSWER 2 OF 71 MEDLINE on STN
ACCESSION NUMBER: 2002053807 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11779212
TITLE: Specificity role of the **streptokinase** C-terminal domain in plasminogen activation.

AUTHOR: Kim Dong Min; Lee Sang Jun; Yoon Suk Kwon; Byun Si Myung
CORPORATE SOURCE: Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), 305-701 Taejon, South Korea.
SOURCE: Biochemical and biophysical research communications, (2002 Jan 11) 290 (1) 585-8.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20020125
Last Updated on STN: 20020226
Entered Medline: 20020225

AB Several pathogenic bacteria secrete plasminogen activator proteins. **Streptokinase** (SKe) produced by Streptococcus **equisimilis** and staphylokinase secreted from Staphylococcus aureus are human plasminogen activators and **streptokinase** (SKu), produced by Streptococcus uberis, is a bovine plasminogen activator. Thus, the fusion proteins among these activators can explain the function of each domain of SKe. Replacement of the SKalpha domain with staphylokinase donated the staphylokinase-like activation activity to SKe, and the SKbetagamma domain played a role of nonproteolytic activation of plasminogen. **Recombinant** SKu also activated human plasminogen by staphylokinase-like activation mode. Because SKu has homology with SKe, the bovine plasminogen activation activities of SKe fragments were checked. SKebetagamma among them had activation activity with bovine plasminogen. This means that the C-terminal domain (gamma-domain) of **streptokinase** determines plasminogen species necessary for activation and converses the ability of substrate recognition to human species.

L26 ANSWER 3 OF 71 MEDLINE on STN
ACCESSION NUMBER: 2000038313 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10569766
TITLE: **Cloning**, **expression**, sequence analysis, and characterization of **streptokinases** secreted by porcine and equine isolates of Streptococcus **equisimilis**.
AUTHOR: Caballero A R; Lottenberg R; Johnston K H
CORPORATE SOURCE: Department of Microbiology, Immunology and Parasitology, Louisiana State University Medical Center, New Orleans, Louisiana 70112, USA.
CONTRACT NUMBER: R01DK45014 (NIDDK)
SOURCE: Infection and immunity, (1999 Dec) 67 (12) 6478-86.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF104300; GENBANK-AF104301
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991220

AB **Streptokinases** secreted by nonhuman isolates of group C streptococci (Streptococcus equi, S. **equisimilis**, and S. zooepidemicus) have been shown to bind to different mammalian plasminogens but exhibit preferential plasminogen activity. The **streptokinase** genes from S. **equisimilis** strains which activated either equine or porcine plasminogen were **cloned**, sequenced, and **expressed** in Escherichia **coli**. The

streptokinase secreted by the equine isolate had little similarity to any known **streptokinases** secreted by either human or porcine isolates. The **streptokinase** secreted by the porcine isolate had limited structural and functional similarities to **streptokinases** secreted by human isolates. Plasminogen activation studies with immobilized (His)(6)-tagged **recombinant streptokinases** indicated that these **recombinant streptokinases** interacted with plasminogen in a manner similar to that observed when **streptokinase** and plasminogen interact in the fluid phase. Analysis of the cleavage products of the **streptokinase** -plasminogen interaction indicated that human, equine, and porcine plasminogens were all cleaved at the same highly conserved site. The site at which **streptokinase** was cleaved to form altered **streptokinase** (Sk*) was also determined. This study confirmed not only the presence of **streptokinases** in nonhuman S. **equisimilis** isolates but also that these proteins belong to a family of plasminogen activators more diverse than previously thought.

L26 ANSWER 4 OF 71 MEDLINE on STN
ACCESSION NUMBER: 1998350778 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9686161
TITLE: **Cloning, expression** and purification of **recombinant streptokinase**: partial characterization of the protein **expressed** in Escherichia **coli**.
AUTHOR: Avilan L; Yarzabal A; Jurgensen C; Bastidas M; Cruz J; Puig J
CORPORATE SOURCE: Laboratorio de Biologia y Medicina Experimental, Facultad de Ciencias, Universidad de Los Andes, Merida, Venezuela.
SOURCE: Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica ... [et al.], (1997 Dec) 30 (12) 1427-30.
Journal code: 8112917. ISSN: 0100-879X.
PUB. COUNTRY: Brazil
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19981020
Last Updated on STN: 19981020
Entered Medline: 19981005

AB We **cloned** the **streptokinase** (STK) gene of Streptococcus **equisimilis** in an **expression** vector of Escherichia **coli** to overexpress the profibrinolytic protein under the control of a tac promoter. Almost all the **recombinant** STK was exported to the periplasmic space and recovered after gentle lysozyme digestion of induced cells. The periplasmic fraction was chromatographed on DEAE Sepharose followed by chromatography on phenyl-agarose. Active proteins eluted between 4.5 and 0% ammonium sulfate, when a linear gradient was applied. Three major STK derivatives of 47.5 kDa, 45 kDa and 32 kDa were detected by Western blot analysis with a polyclonal antibody. The 32-kDa protein formed a complex with human plasminogen but did not exhibit Glu-plasminogen activator activity, as revealed by a zymographic assay, whereas the 45-kDa protein showed a K(m) = 0.70 microM and kcat = 0.82 s-1, when assayed with a chromogen-coupled substrate. These results suggest that these proteins are putative fragments of STK, possibly derived from partial degradation during the export pathway or the purification steps. The 47.5-kDa band corresponded to the native STK, as revealed by peptide sequencing.

L26 ANSWER 5 OF 71 MEDLINE on STN
ACCESSION NUMBER: 96397500 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8804394

TITLE: **Cloning** of heterologous genes specifying detrimental proteins on pUC-derived plasmids in Escherichia **coli**.
AUTHOR: Muller J; van Dijl J M; Venema G; Bron S
CORPORATE SOURCE: Institut fur Molekularbiologie, Friedrich-Schiller-Universitat Jena, Germany.
SOURCE: Molecular & general genetics : MGG, (1996 Aug 27) 252 (1-2) 207-11.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 19961219
Entered Medline: 19961031

AB A system is described that enables the **cloning** of genes specifying detrimental proteins in Escherichia **coli**. The system is based on pUC plasmids and was developed for the **expression** of the Bacillus subtilis csaA gene, which is lethal when **expressed** at high levels. Suppressor strains that tolerate the presence of plasmids for high-level **expression** of csaA were isolated, which contained small cryptic deletion variants of the parental plasmid in high copy numbers. The cryptic plasmids consisted mainly of the pUC replication functions and lacked the csaA region and selectable markers. The co-resident, incompatible, cryptic plasmids enabled the maintenance of the csaA plasmids by reducing their copy number 20-fold, which resulted in a concomitant 3- to 7-fold reduction in the **expression** of plasmid-encoded genes. Strains carrying these cryptic endogenous plasmids proved to be useful for the construction of pUC-based **recombinant** plasmids carrying other genes, such as the **skc** gene of Streptococcus **equisimilis**, which cannot be **cloned** in high copy numbers in E. **coli**. Several strategies to reduce production levels of heterologous proteins specified by plasmids are compared.

L26 ANSWER 6 OF 71 MEDLINE on STN
ACCESSION NUMBER: 96396845 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8803948
TITLE: Structural dissection and functional analysis of the complex promoter of the **streptokinase** gene from Streptococcus **equisimilis** H46A.
AUTHOR: Grafe S; Ellinger T; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Medical microbiology and immunology, (1996 May) 185 (1) 11-7.
Journal code: 0314524. ISSN: 0300-8584.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970131

AB The overlapping tandem promoters of the **streptokinase** gene, P1 and P2, identified previously by S1 nuclease transcript mapping were functionally dissected by mutagenesis of their -10 regions and fused transcriptionally with or without the 202-bp upstream region (USR) to the luciferase reporter gene (luc) from Photinus pyralis to analyze the contribution of the different sequence elements to promoter activity in Escherichia **coli** and the homologous Streptococcus **equisimilis** strain H46A. In E. **coli**, virtually the

entire promoter activity derived from the upstream promoter P1. In S. **equisimilis**, luc **expression** increased in the following order of the involved sequence elements: P2 approximately equal to P2 + USR < P1 < P1 + P2 < P1 + USR < P1 + P2 + USR. This shows that (1) in the homologous system, P1 and P2 alone are extremely weak, (2) in the USR-less arrangement, only the combined core promoters have substantial activity, and (3) the USR stimulates only P1 and the combination of P1 + P2. Thus, the tandem promoters presumably function by mutual contributary action and their full activity strongly depends on the AT-rich and statically bent upstream region. The distinctive feature determining the strength of P1 in both hosts appears to be its extended -10 region which matches the consensus TRTGN established for strong S. pneumoniae and Bacillus subtilis promoters.

L26 ANSWER 7 OF 71 MEDLINE on STN
ACCESSION NUMBER: 96305364 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8706717
TITLE: **Cloning**, sequencing and functional overexpression of the Streptococcus **equisimilis** H46A gapC gene encoding a glyceraldehyde-3-phosphate dehydrogenase that also functions as a plasmin(ogen)-binding protein. Purification and biochemical characterization of the protein.
AUTHOR: Gase K; Gase A; Schirmer H; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: European journal of biochemistry / FEBS, (1996 Jul 1) 239 (1) 42-51.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X97788
ENTRY MONTH: 199609
ENTRY DATE: Entered STN: 19960919
Last Updated on STN: 19990129
Entered Medline: 19960910

AB We previously identified DNA sequences involved in the function of the complex promoter of the **streptokinase** gene from Streptococcus **equisimilis** H46A, a human serogroup C strain known to **express** this gene at a high level. As a prerequisite to understanding possible mechanisms that control the balance between the plasminogen activating and plasmin(ogen) binding capacities of H46A, we describe here its gapC gene encoding glyceraldehyde-3-phosphate dehydrogenase (GraP-DH, EC 1.2.1.12), a glycolytic enzyme apparently transported to the cell surface where it functions as a plasmin(ogen).binding protein. The gapC gene was **cloned** and sequenced and found to code for a 336-amino-acid polypeptide (approximately 35.9 kDa) exhibiting 94.9% sequence identity to the Plr protein from Streptococcus pyogenes shown by others to be capable of plasmin binding [Lottenberg, R., Broder, C. C., Boyle, M. D., Kain, S. J., Schroeder, B. L. & Curtiss, R. III (1992) J. Bacteriol. 174, 5204-5210]. To study the properties of the GapC protein, its gene was inducibly overexpressed in Escherichia **coli** from QIAexpress **expression** plasmids to yield the authentic GapC or (His)6GapC carrying a hexahistidyl N-terminus to permit affinity purification. Both proteins were functionally active, exhibiting specific GraP-DH activities of about 80 kat/mol (approximately 130 U/mg) after purification. Their binding parameters [association (ka) and dissociation (kd) rate constants, and equlibrium dissociation constants (Kd = kd/ka)] for the interaction with human Gluplasminogen and plasmin were determined by real-time biospecific interaction analysis using the Pharmacia BIAcore instrument. For comparative purposes, the commercial GraP-DH from Bacillus stearothermophilus (BstGraP-DH), a nonpathogenic organism, was included in

these experiments. The Kd values for binding of plasminogen to GapC, (His)6GapC and BstGraP-DH were 220 nM, 260 nM and 520 nM, respectively, as compared to 25 nM, 17 nM and 98 nM, respectively, for the binding to plasmin. These data show that both the zymogen and active enzyme possess low-affinity binding sites for the gapC gene product and that the hexahistidyl terminus does not affect its function. Prior limited treatment with plasmin enhanced the subsequent plasminogen binding capacity of all three GraP-DHs, presumably by the exposure of new C-terminal lysine residues for binding to the zymogen.

L26 ANSWER 8 OF 71 MEDLINE on STN
ACCESSION NUMBER: 96200111 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8631718
TITLE: Functional analysis of a relA/spoT gene homolog from Streptococcus **equisimilis**.
AUTHOR: Mechold U; Cashel M; Steiner K; Gentry D; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Journal of bacteriology, (1996 Mar) 178 (5) 1401-11.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960715
Last Updated on STN: 19970203
Entered Medline: 19960703

AB We examined the functional attributes of a gene encountered by sequencing the **streptokinase** gene region of Streptococcus **equisimilis** H46A. This gene, originally called rel, here termed relS. **equisimilis**, is homologous to two related Escherichia **coli** genes, spoT and relA, that function in the metabolism of guanosine 5',3'-polyphosphates [(p)ppGpp]. Studies with a variety of E. **coli** mutants led us to deduce that the highly **expressed** rel S. **equisimilis** gene encodes a strong (p)ppGppase and a weaker (p)ppGpp synthetic activity, much like the spoT gene, with a net effect favoring degradation and no complementation of the absence of the relA gene. We verified that the Rel S. **equisimilis** protein, purified from an E. **coli** relA spoT double mutant, catalyzed a manganese-activated (p)ppGpp 3'-pyrophosphohydrolase reaction similar to that of the SpoT enzyme. This Rel S. **equisimilis** protein preparation also weakly catalyzed a ribosome-independent synthesis of (p)ppGpp by an ATP to GTP 3'-pyrophosphoryltransferase reaction when degradation was restricted by the absence of manganese ions. An analogous activity has been deduced for the SpoT protein from genetic evidence. In addition, the Rel S. **equisimilis** protein displays immunological cross-reactivity with polyclonal antibodies specific for SpoT but not for RelA. Despite assignment of rel S. **equisimilis** gene function in E. **coli** as being similar to that of the native spoT gene, disruptions of rel S. **equisimilis** in S. **equisimilis** abolish the parental (p)ppGpp accumulation response to amino acid starvation in a manner expected for relA mutants rather than spoT mutants.

L26 ANSWER 9 OF 71 MEDLINE on STN
ACCESSION NUMBER: 96001243 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7557478
TITLE: Secretion of **streptokinase** fusion proteins from Escherichia **coli** cells through the hemolysin transporter.
AUTHOR: Kern I; Ceglowski P
CORPORATE SOURCE: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa.
SOURCE: Gene, (1995 Sep 22) 163 (1) 53-7.
Journal code: 7706761. ISSN: 0378-1119.

PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199511
ENTRY DATE: Entered STN: 19951227
Last Updated on STN: 20021008
Entered Medline: 19951108

AB The hemolysin (HlyA) secretion system was used to achieve the sec-independent secretion of **streptokinase** (**Skc**) originating from Streptococcus **equisimilis** into the medium by Escherichia **coli** cells. The in-frame fusions of the **skc** gene, either possessing or lacking a region encoding the signal peptide (SP) with the 3'-end of the hlyA gene of various lengths were analysed. All hybrids retained **Skc** activity. Hybrid proteins devoided of the N-terminal SP, regardless of length of the hlyA secretion signal (62 vs. 194 amino acids), were secreted into the medium by the E. **coli** HlyA transporter at similar levels. Considerable amounts of hybrid proteins were still, however, associated with E. **coli** cells, mainly in the degraded form.

L26 ANSWER 10 OF 71 MEDLINE on STN
ACCESSION NUMBER: 95342169 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7616967
TITLE: Complex transcriptional control of the **streptokinase** gene of Streptococcus **equisimilis** H46A.
AUTHOR: Gase K; Ellinger T; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Molecular & general genetics : MGG, (1995 Jun 25) 247 (6) 749-58.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950905
Last Updated on STN: 19950905
Entered Medline: 19950822

AB On the Streptococcus **equisimilis** H46A chromosome, the divergent coding sequences of the genes for the plasminogen activator **streptokinase** (**skc**) and a leucine-rich protein (lrp), the function of which is unknown, are separated by a 328 bp intrinsically bent DNA region rich in AT tracts. To begin to understand the **expression** control of these two genes, we mapped their transcriptional initiation sites by S1 nuclease analysis and studied the influence of the bent intergenic region on promoter strength, using promoter-reporter gene fusions of **skc**' and lrp' to 'lacZ from Escherichia **coli**. The major transcriptional start sites, in both S. **equisimilis** and E. **coli**, mapped 22 bases upstream of the ATG start site of lrp (G), and 24 and 32 bases upstream of the translational initiation codon of **skc** (A and G, respectively), indicating the existence of two overlapping canonical **skc** promoters arranged in tandem on opposite faces of the helix. The reporter gene fusions were **cloned** in E. **coli** on a vector containing a 1.1 kb fragment of the S. **equisimilis** dexB gene, thus allowing promoter strength to be measured in multiple plasmid-form copies in the heterologous host and in single-copy genomic form following integration into the **skc** region of the homologous host. In S. **equisimilis**, **skc**'-'lacZ was **expressed** about 200-fold more strongly than the corresponding lrp'-'lacZ fusion. In contrast, in E. **coli**, the corresponding levels of **expression** differed by only about 11-fold. Deletion

of the 202 bp bent region upstream of the **skc** and lrp core promoters caused a 13-fold decrease in **skc** promoter activity in S. **equisimilis** but did not alter lrp promoter strength in this host. In contrast, when studied in E. **coli**, this deletion did not alter the strength of the **skc**-double promoter and even increased by 2.4- to 3-fold the activity of the lrp promoter. This comparative promoter analysis shows that **skc** has a complex promoter structure, the activity of which in the homologous genomic environment specifically depends on sequences upstream of the two core promoters. Thus, the **skc** promoter structure resembles that of an array of promoters involved in a transcriptional switch; however, the nature of the potential switch factor(s) remains unknown.

L26 ANSWER 11 OF 71 MEDLINE on STN
ACCESSION NUMBER: 95157528 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7531815
TITLE: Transcription termination of the **streptokinase** gene of Streptococcus **equisimilis** H46A: bidirectionality and efficiency in homologous and heterologous hosts.
AUTHOR: Steiner K; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Molecular & general genetics : MGG, (1995 Feb 6) 246 (3) 374-80.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199503
ENTRY DATE: Entered STN: 19950322
Last Updated on STN: 19960129
Entered Medline: 19950316

AB In Streptococcus **equisimilis** H46A, a hypersymmetrical transcription terminator with bidirectional activity was localized between the translational termination codons of the **streptokinase** gene, **skc**, and the rel-orf1 genes. These two transcription units are oriented towards each other, and under normal conditions the **skc** mRNA level exceeds that of the rel-orf1 genes by a factor of at least 1000. Reporter vectors based on the promoterless cat gene were constructed by transcriptional fusion of **skc** to cat, such that the region between the two genes contained the terminator in **skc** orientation or in rel-orf1 orientation. Additionally, **skc** and cat were fused directly, with deletion of the terminator. The reporter vectors were designed to be capable of being studied either as multicopy plasmids in Escherichia **coli** or in single copy following integration, via **skc**, into the S. **equisimilis** chromosome. Chloramphenicol acetyl transferase (CAT) activity assays in conjunction with determination of chloramphenicol resistance levels and Northern hybridization analysis showed that the terminator is active in either host and orientation. However, termination efficiency was host dependent, with high terminator strength being observed in the homologous streptococcal background and appreciable readthrough occurring in E. **coli**. The extent of transcriptional readthrough was dependent upon terminator orientation, with termination being more efficient in rel-orf1 polarity. The results suggest that, in S. **equisimilis**, transcription of both **skc** and rel-orf1 is efficiently terminated by a common signal, and that these genes are largely protected from convergent transcription, which otherwise would seem to be particularly detrimental to the weakly **expressed** rel-orf1 genes.

L26 ANSWER 12 OF 71 MEDLINE on STN
ACCESSION NUMBER: 94049672 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8232196

TITLE: Genetic organization of the **streptokinase** region of the Streptococcus **equisimilis** H46A chromosome.
AUTHOR: Mechold U; Steiner K; Vettermann S; Malke H
CORPORATE SOURCE: Institute for Molecular Biology, Jena University, Germany.
SOURCE: Molecular & general genetics : MGG, (1993 Oct) 241 (1-2) 129-40.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X72832
ENTRY MONTH: 199312
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 19940117
Entered Medline: 19931215

AB The complete nucleotide sequences of four genes and one open reading frame (ORF1) adjacent to the **streptokinase** gene, **skc**, from Streptococcus **equisimilis** H46A were determined. These genes are encoded on the opposite DNA strand to **skc** and are arranged as follows: dexB-abc-lrp-**skc**-ORF1-rel. The dexB gene, coding for an alpha-glucosidase (M(r) 61,733), and abc, encoding an ABC transporter (M(r) 42,080), are similar to the dexB and msmK genes, respectively, from the multiple sugar metabolism operon of S. mutans. The lrp gene specifies a leucine-rich protein (M(r) 32,302) that has a leucine-zipper motif at its C-terminus. The function of the Lrp protein is not known but appeared to be detrimental when overexpressed in Escherichia **coli**. Although lrp appears not to be an essential gene, as judged by plasmid insertion mutagenesis, it is conserved in all streptococcal strains carrying a **streptokinase** gene. The rel gene showed significant homology to the E. **coli** relA and spoT genes involved in the stringent response to amino acid deprivation. Multiple alignment of the amino acid sequences of Rel (M(r) 83,913), RelA and SpoT revealed 59.4% homology of the primary structures. Northern hybridization analyses of the genes in the **skc** region showed **skc** to be transcribed most abundantly. In addition to transcripts for **skc**, monocistronic mRNAs were detected for all three genes divergently transcribed from **skc**. Although there was also some read-through transcription from lrp into abc, and from abc into dexB, the transcription pattern suggests a high degree of transcriptional and functional independence not only of **skc** but also abc and dexB. Prominent structural features in intergenic regions included a static DNA bending locus located upstream and a putative bidirectional transcription terminator downstream of **skc**.

L26 ANSWER 13 OF 71 MEDLINE on STN
ACCESSION NUMBER: 92039051 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1937032
TITLE: Isolation, sequence and **expression** in Escherichia **coli**, Bacillus subtilis and Lactococcus lactis of the DNase (streptodornase)-encoding gene from Streptococcus **equisimilis** H46A.
AUTHOR: Wolinowska R; Ceglowski P; Kok J; Venema G
CORPORATE SOURCE: Department of Pharmaceutical Microbiology, Medical Academy, Warsaw, Poland.
SOURCE: Gene, (1991 Sep 30) 106 (1) 115-9.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M59725; GENBANK-M59726; GENBANK-M59727; GENBANK-M59728; GENBANK-M63990; GENBANK-S61507; GENBANK-S63856; GENBANK-S63863; GENBANK-S65020;

GENBANK-S65060; GENBANK-X17241
ENTRY MONTH: 199112
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19920124
Entered Medline: 19911223

AB A partial library of BclI-generated chromosomal DNA fragments from Streptococcus **equisimilis** H64A (Lancefield Group C) was constructed in Escherichia **coli**. **Clones** displaying either **streptokinase** or deoxyribonuclease (streptodornase; SDC) activities were isolated. The gene (sdc) **expressing** the SDC activity was allocated on the 1.1-kb AccI DNA subfragment. Sequence analysis of this DNA fragment revealed the presence of one open reading frame, which could encode a protein of 36.8 kDa. The N-terminal portion of the deduced protein exhibited features characteristic of prokaryotic signal peptides. The sdc gene was **expressed** in E. **coli** , Bacillus subtilis and Lactococcus lactis. As observed for S. **equisimilis**, in the heterologous Gram + hosts, at least part of the SDC protein was secreted into the medium.

L26 ANSWER 14 OF 71 MEDLINE on STN
ACCESSION NUMBER: 90172183 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2625666
TITLE: Sequence-directed DNA bending upstream of the **streptokinase** promoter.
AUTHOR: Muller J; Malke H
CORPORATE SOURCE: Akademie der Wissenschaften der DDR.
SOURCE: Journal of basic microbiology, (1989) 29 (9) 611-6.
Journal code: 8503885. ISSN: 0233-111X.
PUB. COUNTRY: GERMANY, EAST: German Democratic Republic
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199004
ENTRY DATE: Entered STN: 19900601
Last Updated on STN: 19900601
Entered Medline: 19900409

AB A 450-base pair (bp) HinfI restriction fragment from the chromosome of Streptococcus **equisimilis** H46A contains the early coding region of the **streptokinase** gene (**skc**), the **skc** promoter, and a stretch of DNA 5' to the--35 region of the **skc** promoter. Two-dimensional polyacrylamide (PA) gel electrophoresis at two different temperatures showed that this fragment migrates anomalously slowly on PA gels, suggesting the existence of a bent DNA conformation. Inspection of the nucleotide sequence confirmed this suggestion by revealing numerous oligomeric dA.dT tracts, some of which are in phase with the helix screw. Computer analysis of the sequence predicted the existence of two bending loci, one of which is located upstream of the **skc** promoter. In addition to showing DNA bending, the 450-bp HinfI fragment contains multiple 13-bp sequences homologous to the Escherichia **coli** integration host factor DNA-binding consensus sequence. Insertion of IS1 into a site immediately upstream of the--35 region decreased the **expression** level of **skc** in E. **coli**, suggesting that DNA conformation upstream of the promoter has a role in **skc expression**.

L26 ANSWER 15 OF 71 MEDLINE on STN
ACCESSION NUMBER: 88302119 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3043172
TITLE: Tripartite **streptokinase** gene fusion vectors for gram-positive and gram-negative procaryotes.
AUTHOR: Klessen C; Schmidt K H; Ferretti J J; Malke H
CORPORATE SOURCE: Academy of Sciences of the GDR, Central Institute of Microbiology and Experimental Therapy, Jena.
SOURCE: Molecular & general genetics : MGG, (1988 May) 212 (2)

295-300.
Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198809
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19900308
Entered Medline: 19880921

AB A specific 1,596 bp HincII fragment ('**skc**) from the chromosome of Streptococcus **equisimilis** contains an active **streptokinase** (SK) gene (**skc**) lacking, in addition to the **expression** signals, codons 1 through 39 of wild-type **skc** but retaining the remainder of the **skc** coding sequence together with the transcription terminator. Using this fragment as an indicator gene, we constructed two types of vectors which in appropriate hosts resulted in the synthesis of SK fusion proteins after insertional activation of '**skc**. The first type are open reading frame (ORF) vectors in which '**skc** was inserted into pUC18 out of frame with respect to lacZ', thus conferring an SK-negative phenotype. Any DNA fragments representing ORFs inserted between the lacZ' **expression** signals and '**skc** such that the **skc** reading frame was restored resulted in the production of tripartite proteins which exhibited SK activity. The second type of vector, which functioned in both gram-positive and gram-negative bacteria, used the streptococcal speA **expression** and secretion signals in front of the ORF to activate '**skc** insertionally. Using a large fragment from the chymosin gene as the target sequence, the usefulness of these vectors for studying foreign gene **expression** in streptococci as well as Escherichia **coli** was demonstrated.

L26 ANSWER 16 OF 71 MEDLINE on STN
ACCESSION NUMBER: 84221999 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6374659
TITLE: **Streptokinase: cloning, expression**, and excretion by Escherichia **coli**.
AUTHOR: Malke H; Ferretti J J
CONTRACT NUMBER: AI 9304 (NIAID)
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1984 Jun) 81 (11) 3557-61.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198407
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19970203
Entered Medline: 19840724

AB Genomic DNA from Streptococcus **equisimilis** strain H46A was **cloned** in Escherichia **coli** by using the bacteriophage lambda replacement vector L47 and an in vitro packaging system. A casein/plasminogen overlay technique was used to screen the phage bank for **recombinants** carrying the **streptokinase** gene (**skc**). The gene was present with a frequency of 1 in 836 **recombinants**, and 10 independent **clones** containing **skc** were isolated and physically characterized. One **recombinant clone** was used to subclone **skc** in E. **coli** plasmid vectors. Plasmid pMF2 [10.4 kilobases (kb)] consisting of pACYC184 with a 6.4-kb H46A DNA fragment in the EcoRI site and pMF5 (6.9 kb) carrying a 2.5-kb fragment in the Pst I site of pBR322 were among the **recombinant** plasmids determining

streptokinase production in three different E. **coli** host strains. **Expression** of **skc** was independent of its orientation in either vector, indicating that its own promoter was present and functional in E. **coli**. However, **expression** in pBR322 was more efficient in one orientation than in the other, suggesting that one or both of the bla gene promoters contributed to **skc expression**. Several lines of evidence, including proof obtained by the immunodiffusion technique, established the identity of E. **coli streptokinase**. Testing cell-free culture supernatant fluids, osmotic shock fluids, and sonicates of osmotically shocked cells for **streptokinase** activity revealed the substance to be present in all three principal locations, indicating that E. **coli** cells were capable of releasing substantial amounts of **streptokinase** into the culture medium.

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ACCESSION NUMBER: 2005441717 EMBASE
TITLE: Heberkinasa: **Recombinant streptokinase** [9].
AUTHOR: Hernandez L.; Martinez Y.; Quintana M.; Besada V.; Martinez E.
CORPORATE SOURCE: L. Hernandez, Production Division, Centro de Ingenieria Genetica Y Biotecnologia, Ave 31 e/ 158 y 190, Cubanacan, Playa, Habana 0600, Cuba. luciano.hernandez@cigb.edu.cu
SOURCE: European Heart Journal, (2005) Vol. 26, No. 16, pp. 1691. Refs: 4
ISSN: 0195-668X CODEN: EHJODF
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Letter
FILE SEGMENT: 018 Cardiovascular Diseases and Cardiovascular Surgery
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
ENTRY DATE: Entered STN: 20051020
Last Updated on STN: 20051020

L26 ANSWER 18 OF 71 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2002110457 EMBASE
TITLE: Can imperfections help to improve bioreactor performance?.
AUTHOR: Patnaik P.R.
CORPORATE SOURCE: P.R. Patnaik, Institute of Microbial Technology, Sector 39-A, Chandigarh-160 036, India. pratap@imtech.res.in
SOURCE: Trends in Biotechnology, (1 Apr 2002) Vol. 20, No. 4, pp. 135-137.
Refs: 22
ISSN: 0167-7799 CODEN: TRBIDM
PUBLISHER IDENT.: S 0167-7799(01)01922-9
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
027 Biophysics, Bioengineering and Medical Instrumentation
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20020404
Last Updated on STN: 20020404

AB Pilot-scale and larger bioreactors differ from small laboratory-scale reactors in terms of a greater occurrence of noise and incomplete mixing of the broth. Conventional control tries to induce good mixing and to filter out the noise as completely as possible. As such an 'ideal' operation is difficult to achieve, recent work has tried to exploit the non-ideal features to improve the performance. Using artificial neural

networks, the degree of mixing, the extent of filtering of noise and the distribution of plasmid copy number (in a **recombinant** fermentation) can be controlled effectively on-line. This strategy generates better productivities than well-mixed noise-free operations, which suggests that deviations from ideal behaviour should be gainfully harnessed and not suppressed.

L26 ANSWER 19 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:96119 BIOSIS
DOCUMENT NUMBER: PREV200000096119
TITLE: Two **streptokinase** genes are **expressed** with different solubility in Escherichia **coli** W3110.
AUTHOR(S): Pupo, Elder [Reprint author]; Baghbaderani, Behnam A.; Lugo, Victoria; Fernandez, Julio; Paez, Rolando; Torrens, Isis
CORPORATE SOURCE: Biopharmaceutical Development Division, Center for Genetic Engineering and Biotechnology, Havana, Cuba
SOURCE: Biotechnology Letters, (Dec., 1999) Vol. 21, No. 12, pp. 1119-1123. print.
CODEN: BILED3. ISSN: 0141-5492.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 15 Mar 2000
Last Updated on STN: 3 Jan 2002
AB The **streptokinase** (SK) gene from S. **equisimilis** H46A (ATCC 12449) was **cloned** in E. **coli** W3110 under the control of the tryptophan promoter. The **recombinant** SK, which represented 15% of total cell protein content, was found in the soluble fraction of disrupted cells. The solubility of this SK notably differed from that of the product of the SK gene from S. **equisimilis** (ATCC 9542) which had been **cloned** in E. **coli** W3110 by using similar **expression** vector and cell growth conditions, and occurred in the form of inclusion bodies.

L26 ANSWER 20 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:88244 BIOSIS
DOCUMENT NUMBER: PREV200000088244
TITLE: Hydrodynamic radii of native and denatured proteins measured by pulse field gradient NMR techniques.
AUTHOR(S): Wilkins, Deborah K.; Grimshaw, Shaun B.; Receveur, Veronique; Dobson, Christopher M.; Jones, Jonathan A.; Smith, Lorna J. [Reprint author]
CORPORATE SOURCE: Oxford Centre for Molecular Sciences, New Chemistry Laboratory, University of Oxford, South Parks Road, Oxford, OX1 3QT, UK
SOURCE: Biochemistry, (Dec. 14, 1999) Vol. 38, No. 50, pp. 16424-16431. print.
CODEN: BICHAW. ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Mar 2000
Last Updated on STN: 3 Jan 2002
AB Pulse field gradient NMR methods have been used to determine the effective hydrodynamic radii of a range of native and nonnative protein conformations. From these experimental data, empirical relationships between the measured hydrodynamic radius (Rh) and the number of residues in the polypeptide chain (N) have been established; for native folded proteins Rh = 4.75N 0.29ANG and for highly denatured states Rh = 2.21N 0.57ANG. Predictions from these equations agree well with experimental data from dynamic light scattering and small-angle X-ray or neutron scattering studies reported in the literature for proteins ranging in size

from 58 to 760 amino acid residues. The predicted values of the hydrodynamic radii provide a framework that can be used to analyze the conformational properties of a range of nonnative states of proteins. Several examples are given here to illustrate this approach including data for partially structured molten globule states and for proteins that are unfolded but biologically active under physiological conditions. These reveal evidence for significant coupling between local and global features of the conformational ensembles adopted in such states. In particular, the effective dimensions of the polypeptide chain are found to depend significantly on the level of persistence of regions of secondary structure or features such as hydrophobic clusters within a conformational ensemble.

L26 ANSWER 21 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:317596 BIOSIS
DOCUMENT NUMBER: PREV199800317596
TITLE: Effect of signal peptide changes on the extracellular processing of **streptokinase** from Escherichia **coli**: Requirement for secondary structure at the cleavage junction.
AUTHOR(S): Pratap, J.; Dikshit, K. L. [Reprint author]
CORPORATE SOURCE: Inst. Microbial Technology, Sector 39-A, Chandigarh 160 036, India
SOURCE: Molecular and General Genetics, (May, 1998) Vol. 258, No. 4, pp. 326-333. print.
CODEN: MGGEAE. ISSN: 0026-8925.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 22 Jul 1998
Last Updated on STN: 10 Sep 1998

AB **Streptokinase** (SK), an extracellular protein from Streptococcus **equisimilis**, is secreted post-translationally by Escherichia **coli** using both its native and E. **coli**-derived transport signals. In this communication we report that cleavage specificity of signal peptidase I, and thus efficiency of secretion, varies in E. **coli** when SK export is directed by different transport signals. The native (+ 1) N-terminus of mature SK was retained when it was transported under the control of its own, PelB or LamB signal peptide. However, when translocation of SK was controlled by the OmpA or MalE signal peptide, Ala2 of mature SK was preferred as a cleavage site for the pre-SK processing. Our results indicate that compatibility of the leader peptide with the mature sequences of SK, which fulfils the requirement for a given secondary structure within the cleavage region, is essential for maintaining the correct processing of pre-SK. An OmpA-SK fusion, which results in the deletion of two N-terminal amino acid residues of mature SK, was further studied with respect to the recognition of alternative cleavage site in E. **coli**. The alanine at +2 in mature SK was changed to glycine or its relative position was changed to +3 by introducing a methionine residue at the +1 position. Both alterations resulted in the correct cleavage of pre-SK at the original OmpA fusion site. In contrast, introduction of an additional alanine at +4, creating three probable cleavage sites (Ala-x-Ala-x-Ala-x-Ala), resulted in the recognition of all three target sites for cleavage, with varying efficiency. The results indicate that the nature of the secondary structure generated at the cleavage junction of pre-SK, resulting from the fusion of different signal peptides, modulates the cleavage specificity of signal peptidase I during extracellular processing of SK. Based on these findings it is proposed that flexibility in the interaction of the active site of signal peptidase I with the cleavage sites of signal peptides may occur when it encounters two or more juxtaposed cleavage sites. Preference for one cleavage site over another, then, may depend on fulfillment of secondary structure requirements in the vicinity of the pre-protein cleavage junction.

L26 ANSWER 22 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1995:547506 BIOSIS
DOCUMENT NUMBER: PREV199698561806
TITLE: High-level **expression** and secretion of **streptokinase** in Escherichia **coli**.
AUTHOR(S): Ko, Jae Hyeong; Park, Do Deun; Kim, Il Chul; Lee, Si Hyoung; Byun, Si Myung [Reprint author]
CORPORATE SOURCE: Dep. BioSci., Korea Advanced Inst. Sci. Technol., 373-1, Kusung-dong, Yusung-ku, Taejeon 305-701, South Korea
SOURCE: Biotechnology Letters, (1995) Vol. 17, No. 10, pp. 1019-1024.
CODEN: BILED3. ISSN: 0141-5492.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 31 Dec 1995
Last Updated on STN: 31 Dec 1995
AB The high-level **expression** plasmid for **streptokinase**, pSK100, has been constructed. It contains a tac promoter, an ompA signal sequence, a **streptokinase** structural gene(**skc**) and a rrnBT1T2 transcription terminator. E. **coli** JM 109 carrying pSK100 produced about 5,000IU of **streptokinase** per 1 ml of LB-ampicillin media. About 95% of the **expressed** **streptokinase** was secreted into the periplasmic and extracellular fractions. The **recombinant** **streptokinase** in high yield and purity may be a potential alternative source for the therapeutic agent.

L26 ANSWER 23 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1992:340953 BIOSIS
DOCUMENT NUMBER: PREV199243030503; BR43:30503
TITLE: **STREPTOKINASE** MUTATIONS AFFECTING **SKC** **EXPRESSION** IN HOMOLOGOUS AND HETEROLOGOUS HOSTS.
AUTHOR(S): MECHOLD U [Reprint author]; MULLER J; MALKE H
CORPORATE SOURCE: CENTRAL INST MICROBIOL EXP THERAPY, JENA D-6900, GER
SOURCE: Zentralblatt fuer Bakteriologie Supplement, (1992) pp. 336-338. OREFICI, G. (ED.). ZENTRALBLATT FUER BAKTERIOLOGIE SUPPLEMENT, 22. NEW PERSPECTIVES ON STREPTOCOCCI AND STREPTOCOCCAL INFECTIONS; (INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY, 22. NEW PERSPECTIVES ON STREPTOCOCCI AND STREPTOCOCCAL INFECTIONS); XI LANCEFIELD INTERNATIONAL SYMPOSIUM ON STREPTOCOCCI AND STREPTOCOCCAL DISEASES, SIENA, ITALY, SEPTEMBER 10-14, 1990. XIX+569P. GUSTAV FISCHER VERLAG: STUTTGART, GERMANY; NEW YORK, NEW YORK, USA. ILLUS.
Publisher: Series: Zentralblatt fuer Bakteriologie Supplement.
ISSN: 0941-018X. ISBN: 3-437-11362-3, 1-56081-333-4.
DOCUMENT TYPE: Book
Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 16 Jul 1992
Last Updated on STN: 16 Jul 1992

L26 ANSWER 24 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1991:513401 BIOSIS
DOCUMENT NUMBER: PREV199141114116; BR41:114116
TITLE: **EXPRESSION** AND PROPERTIES OF HYBRID **STREPTOKINASES** EXTENDED BY AMINO-TERMINAL PLASMINOGEN KRINGLE DOMAINS.

AUTHOR(S): MALKE H [Reprint author]; FERRETTI J J
CORPORATE SOURCE: DEP MICROBIOL IMMUNOL, UNIV OKLA HEALTH SCI CENTER, OKLAHOMA CITY, OKLA 73190, USA
SOURCE: (1991) pp. 184-189. DUNNY, G. M., P. P. CLEARY AND L. L. MCKAY (ED.). GENETICS AND MOLECULAR BIOLOGY OF STREPTOCOCCI, LACTOCOCCI, AND ENTEROCOCCI; THIRD INTERNATIONAL ASM (AMERICAN SOCIETY FOR MICROBIOLOGY) CONFERENCE, MINNEAPOLIS, MINNESOTA, USA, JUNE 6-9, 1990. VIII+310P. AMERICAN SOCIETY FOR MICROBIOLOGY: WASHINGTON, D.C., USA. ILLUS.
ISBN: 1-55581-034-9.
DOCUMENT TYPE: Book
Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 14 Nov 1991
Last Updated on STN: 14 Nov 1991

L26 ANSWER 25 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1987:499919 BIOSIS
DOCUMENT NUMBER: PREV198733127633; BR33:127633
TITLE: **STREPTOKINASE EXPRESSION** OF ALTERED FORMS.
AUTHOR(S): MALKE H [Reprint author]; LORENZ D; FERRETTI J J
CORPORATE SOURCE: ACAD SCI GER DEMOCRATIC REPUBLIC, CENT INST MICROBIOL AND EXP THERAPY, DDR-69 JENA, GDR
SOURCE: (1987) pp. 143-149. FERRETTI, J. J. AND R. CURTISS, III (ED.). STREPTOCOCCAL GENETICS; SECOND ASM (AMERICAN SOCIETY FOR MICROBIOLOGY) CONFERENCE, MIAMI, FLORIDA, USA, MAY 21-24, 1986. VIII+300P. AMERICAN SOCIETY FOR MICROBIOLOGY: WASHINGTON, D.C., USA. ILLUS.
ISBN: 0-914826-93-X.
DOCUMENT TYPE: Book
Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 27 Nov 1987
Last Updated on STN: 27 Nov 1987

L26 ANSWER 26 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1987:277792 BIOSIS
DOCUMENT NUMBER: PREV198784018831; BA84:18831
TITLE: MOLECULAR **CLONING** OF **STREPTOKINASE** GENE FROM STREPTOCOCCUS-**EQUISIMILIS** AND ITS **EXPRESSION** IN ESCHERICHIA-**COLI**.
AUTHOR(S): ROH D C [Reprint author]; KIM J H; PARK S K; LEE J W; BYRUN S M
CORPORATE SOURCE: DEP BIOLOGICAL SCIENCE AND ENGINEERING, KOREA ADVANCED INST SCIENCE AND TECHNOLOGY KAIST , PO BOX 150 CHONGRYANG, SEOUL 131, KOREA
SOURCE: Korean Biochemical Journal, (1986) Vol. 19, No. 4, pp. 391-398.
CODEN: KBCJAK. ISSN: 0368-4881.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 19 Jun 1987
Last Updated on STN: 19 Jun 1987
AB The streptococcal genomic DNA digested with Pst I was **cloned** in E. **coli** HB101. The overlay technique of casein/plasminogen was used to screen the **clones** for **recombinants** carrying the **streptokinase** gene. The insert size of the plasmid carrying

the **streptokinase** gene was a 2.5, 4.3, and 5.8 Kb, respectively. The restriction maps of all three hybrid plasmids were constructed by digestion with Pst I, Pvu II, Sal I, Hind III, Ava I, BamH I, and Cla I. For the identification of **cloned** gene, **streptokinase** was highly purified from S. **equisimilis** by the methods of gel chromatography and isoelectric focusing and rabbits were immunized with this purified **streptokinase**. Several lines of evidence, including proof obtained by the immunodiffusion technique, established that the enzyme from E. **coli** was identical to that from S. **equisimilis**. In the E. **coli** cell culture, we found the activity of **streptokinase** in all three principal locations of the cell. More than 50% were existed in the intracellular space.

L26 ANSWER 27 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1984:100214 BIOSIS
DOCUMENT NUMBER: PREV198427016706; BR27:16706
TITLE: HYBRIDIZATION OF A **CLONED** GROUP C STREPTOCOCCAL STREPTO KINASE GENE WITH DNA FROM OTHER STREPTOCOCCAL SPECIES.
AUTHOR(S): HUANG T-T [Reprint author]; WEEKS C R; MALKE H; FERRETTI J J
CORPORATE SOURCE: UNIV OKLA HEALTH SCI CENT, OKLAHOMA CITY, OKLA, USA
SOURCE: Abstracts of the Annual Meeting of the American Society for Microbiology, (1984) Vol. 84, pp. ABSTRACT D75.
Meeting Info.: 84TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, ST. LOUIS, MO., USA, MAR. 4-9, 1984.
ABSTR ANNU MEET AM SOC MICROBIOL.
CODEN: ASMACK. ISSN: 0094-8519.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH

L26 ANSWER 28 OF 71 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1984:92832 BIOSIS
DOCUMENT NUMBER: PREV198427009324; BR27:9324
TITLE: STREPTO KINASE **CLONING EXPRESSION** AND EXCRETION BY ESCHERICHIA-**COLI**.
AUTHOR(S): MALKE H [Reprint author]; FERRETTI J J
CORPORATE SOURCE: ACAD SCI GDR, JENA, GDR
SOURCE: Abstracts of the Annual Meeting of the American Society for Microbiology, (1984) Vol. 84, pp. ABSTRACT D97.
Meeting Info.: 84TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, ST. LOUIS, MO., USA, MAR. 4-9, 1984.
ABSTR ANNU MEET AM SOC MICROBIOL.
CODEN: ASMACK. ISSN: 0094-8519.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH

L26 ANSWER 29 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1994-07329 BIOTECHDS
TITLE: DNA encoding a plasminogen binding protein; **recombinant streptokinase** fragment production using new vector plasmid pMAL and a monoclonal antibody for use in myocardial infarction therapy
PATENT ASSIGNEE: Gen.Hosp.Boston; Univ.Harvard
PATENT INFO: WO 9407992 14 Apr 1994
APPLICATION INFO: WO 1993-US9502 5 Oct 1993
PRIORITY INFO: US 1993-128299 29 Sep 1993; US 1992-956692 5 Oct 1992
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1994-135561 [16]

AB DNA (I) encoding a **streptokinase** fragment (II) of residues 14-414 of a disclosed protein sequence is claimed. (II) does not contain residues 244-352, but may contain residues 1-352, 120-352, 244-414 or 244-352 of the protein sequence. Also claimed are: (1) an **expression** vector containing (I); (2) a host cell transformed with the vector of (1); (3) (II) encoded by (I); (4) detecting plasminogen in a biological sample by contacting the sample with (II) and detecting any (II)-plasminogen complex formed; (5) a method for assaying (II) for antigenicity involving contacting (II) with a monoclonal antibody specific for a distinct epitope of **streptokinase** and determining whether the fragments bind to the MAb and, optionally, also whether the fragments can activate plasminogen in the presence of the MAb; (6) a method for myocardial infarction therapy involving administering (II) to a patient; and (7) a monoclonal antibody specific for a distinct epitope of **streptokinase**. In an example, (II) genes from Streptococcus **equisimilis** were fused with maltose binding protein genes and **expressed** in Escherichia **coli** using plasmid pMAL. (62pp)

L26 ANSWER 30 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1992-13545 BIOTECHDS
TITLE: High level **expression** of **streptokinase** in Escherichia **coli**;
gene **cloning**, **expression** and purification of thrombolytic protein
AUTHOR: Estrada M P; Hernandez L; Perez A; Rodriguez P; *de la Fuente J; Herrera L
LOCATION: Mammalian Cell Genetics Division, Centro de Ingenieria Genetica y Biotecnologia, P.O. Box 6162, Havana 6, Cuba.
SOURCE: Bio/Technology; (1992) 10, 10, 1138-42
CODEN: BTCHDA
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **streptokinase** (SK) gene was isolated by the polymerase chain reaction from Streptomyces **equisimilis** ATCC 9542. The 5-amplification primer introduced an ATG codon for translation initiation in Escherichia **coli**. The amplified fragment, which lacked the signal peptide sequence, was digested with BamHI, inserted into vector plasmid pTrp (to obtain plasmid pEKG-3 containing the SK gene under the control of a trp promoter), and used to transform E. **coli** HB101 cells. The DNA sequence of the SK gene region contained 5 differences at the amino acid level with respect to the reported SK protein. Plasmid pEKG-3 was introduced into E. **coli** K-12 strain W3110 for **expression**. The trp promoter was induced, and maximal SK **expression** was obtained after 14 hr, at which time the plasmid copy number reached 420 copies/cell. The **recombinant** SK was found in the cell cytosol and constituted 25% of total cell protein. It was purified by affinity chromatography using acylated human plasminogen coupled to Sepharose-4B, and ionexchange chromatography on DEAE-Sephacel. The **recombinant** product and natural SK had equivalent biological activities. (38 ref)

L26 ANSWER 31 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1992-03808 BIOTECHDS
TITLE: Method for the isolation and **expression** of a gene encoding **streptokinase**;
Streptococcus **equisimilis** gene **cloning** and vector plasmid pEKG3, plasmid pPESKC-4 and plasmid pPISKC-6 **expression** in Escherichia **coli** or Pichia pastoris
PATENT ASSIGNEE: Cent.Ing.Genet.Biotecnol.
PATENT INFO: AU 9178101 28 Nov 1991
APPLICATION INFO: AU 1991-78101 31 May 1991
PRIORITY INFO: CU 1990-90 23 May 1990

DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1992-024716 [04]
AB A new method for the isolation and **expression** of a gene, **SKC-2**, encoding Streptococcus **equisimilis** C (ATCC 9542) **streptokinase** comprises (i) gene amplification from synthetic oligonucleotides SK1, SK2 and SK3 (specified DNA sequence), (ii) **cloning SKC-2** in bacteria (preferably Escherichia **coli**) with or without a signal peptide; and (iii) intra- or extracellular **expression** in yeast (preferably Pichia pastoris), with the transformed microorganism displaying a high stability and level of **expression**. The following are also claimed: (1) plasmid pEKG3 containing **SKC-2** inserted between the trp promoter and the phage T4 terminator for **expression** in bacteria; (2) plasmid pPESKC-4 and plasmid pPISKC-6, obtained by insertion of **SKC-2** in the yeast **expression** vectors plasmid pPS-7 and plasmid pNAO, respectively, for extra- or intracellular **expression**; (3) transformed microorganisms displaying high levels of **SKC-2** gene **expression**, good viability and cellular stability; (4) the product resulting from **expression** of the **SKC-2** gene in bacteria and yeast; (5) **recombinant** DNA comprising the **SKC-2** DNA sequence; and (6) the **expression** product of the **recombinant** DNA. (28pp)

L26 ANSWER 32 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1991-01878 BIOTECHDS
TITLE: Constructing vector for detecting **expression** of foreign genes;
by inserting element containing **expression** unit, **streptokinase** gene and restriction sites, allowing in frame gene insertion; pro-chymosin, beta-galactosidase production
PATENT ASSIGNEE: Akad.Wiss.DDR
PATENT INFO: DD 279900 20 Jun 1990
APPLICATION INFO: DD 1987-306609 3 Sep 1987
PRIORITY INFO: DD 1987-306609 3 Sep 1987
DOCUMENT TYPE: Patent
LANGUAGE: German
OTHER SOURCE: WPI: 1990-342373 [46]
AB Construction of vectors for detecting heterologous gene **expression** comprises: i. incorporating (in order from N terminus) into a **cloning** vector, a polylinker or restriction site (RS1); **expression** or **expression** secretion unit (Escherichia **coli** lac operon or exotoxin A gene of phage T12 from Streptococcus pyogenes; polylinker or restriction site (RS2); **streptokinase** (SK) structural gene (from Streptococcus **equisimilis** H46A, particularly a 1596 bp HindIII fragment from plasmid pMF5), a polylinker or restriction site (RS3); ii. inserting a foreign gene, X, without a promoter into polylinker or RS2; iii. the resulting detection vector, encoding for an X-SK fusion product, is used to transform microbial receptor cells; and iv. subjecting **recombinant clones** to a plasminogen-milk agar (PMA) overlaying test. Preferably, the vector is a bacterial plasmid or an M13 E. **coli** phage vector. X is a pro-chymosin gene (plasmid pHRW400 or plasmid pHRW500), human interferon-alpha-1, or beta-galactosidase (EC-3.2.1.23, from a pUC plasmid). Recipients are E. **coli** JM101, Streptococcus sanguis Challis 6 or Streptococcus lactis MG1363. (14pp)

L26 ANSWER 33 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1990-02600 BIOTECHDS
TITLE: Site-specific alteration of Gly-24 in **streptokinase** : its effect on plasminogen activation;

site-directed mutagenesis effect on plasminogen-activator activity; gene **cloning** and **expression** in Escherichia **coli**

AUTHOR: Lee B R; Park S K; Kim J H; *Byun S M

LOCATION: Department of Biological Science and Engineering, Korea Advanced Institute of Science and Technology (KAIST), P.O. Box 150, Cheongryang, Seoul, Korea.

SOURCE: Biochem.Biophys.Res.Commun.; (1989) 165, 3, 1085-90
CODEN: BBRCA9

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Oligonucleotide site-directed mutagenesis was performed to replace Gly-24 of Streptococcus **equisimilis** (ATCC 9542) **streptokinase** with His, Glu, or Ala. The **streptokinase** gene was **cloned**, subjected to mutagenesis for removal of the RsaI site, **cloned** into vector plasmid pKS601 under the control of the trp promoter and used to transform Escherichia **coli** C600. The **recombinant** proteins were purified by DEAE-cellulose and Sephadex-G150 chromatography. Substitutions with either His or Glu gave almost complete loss of **streptokinase** activity but **streptokinase** replaced with Ala retained its activity. Although **streptokinases** with His-24 or Glu-24 bound normally to human plasminogen, they did not generate active plasmin, whereas those with Ala-24 or Gly-24 generated active plasmin. The results indicate that the small, uncharged alkyl group side chain on the 24th amino acid residue of **streptokinase** is indispensable for the activity of the human plasminogen-**streptokinase** complex. A charged amino acid in position 24 disrupts beta-sheet formation and prevents **streptokinase** from adopting the orientation required for plasminogen activation. (26 ref)

L26 ANSWER 34 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1985-08513 BIOTECHDS

TITLE: Production of **streptokinase**; by cultivation of Escherichia **coli** ATCC 39613 containing **recombinant** plasmid PMF1

PATENT ASSIGNEE: Phillips-Petrol.

PATENT INFO: AU 8433859 18 Apr 1985

APPLICATION INFO: AU 1984-33859 5 Oct 1984

PRIORITY INFO: US 1984-585417 2 Mar 1984; DD 1983-255523 10 Oct 1983

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 1985-135032 [23]

AB A **recombinant** vector plasmid pMF1 for the transformation of a host to produce **streptokinase** is new. The vector contains a polydeoxyribonucleotide fragment insert which codes for the synthesis and secretion of **streptokinase**. The transformant microorganism is preferably Escherichia **coli** HB101 and the vector, plasmid pBR322. The fragment coding for **streptokinase** synthesis and secretion is derived from a microorganism of the genus Streptococcus, especially Streptococcus **equisimilis** strain H46A and may have restriction endonuclease cleavage sites at the terminii e.g. it has 7400 bp and the cleavage sites are for HindIII. The **recombinant** vector is obtained by digestion of a vector with a restriction endonuclease to give linear DNA. This DNA is ligated to the **streptokinase** fragment to give the **recombinant**. This fragment is obtained by digestion of Streptococcus equilimilis with the same restriction endonuclease, especially Pst I, as is used to digest the initial vector. **Streptokinase** can be produced and isolated for use as a thrombolytic agent to facilitate the in vivo lysis or dissolution of blood clots. (28pp)

L26 ANSWER 35 OF 71 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1984-03868 BIOTECHDS

TITLE: **Streptokinase: cloning expression** and excretion by E.**coli**; using Streptococcus **equisimilis** genomic DNA (conference abstract)
AUTHOR: Malke H; Ferretti J J
LOCATION: Acad. Sci. GDR, Jena, DDR.
SOURCE: Abstr.Annu.Meet.Am.Soc.Microbiol; (1984) 84 Meet., 67
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Genomic DNA from Streptococcus **equisimilis** H46A (group C) was **cloned** into Escherichia **coli** using the lambda replacement vector L47 and an in vitro packaging system. The phage bank was screened for **recombinants** containing the **streptokinase** (**skc**) gene by the casein-plasminogen overlay technique. 10 Independent **clones** containing the **skc** gene were isolated and one was used to subclone the **skc** gene into E.**coli** plasmid vectors pBR322 and pACYC184. Plasmid pMF2 and pMF5 were among the **recombinant** plasmids determining **streptokinase** production in 3 different E. **coli** host strains. **Expression** of **skc** was independent of its orientation in either vector, indicating that its own promoter was present and functional in E.**coli**. Analysis of cell free culture supernatant fluids, osmotic shockates, and sonicates of osmotically shocked cells for **streptokinase** activity revealed the substance to be present in all 3 locations, indicating that E. **coli** cells were capable of releasing substantial amounts of **streptokinase** into the culture medium. (0 ref)

L26 ANSWER 36 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:656911 SCISEARCH
THE GENUINE ARTICLE: 837ZE
TITLE: Control of **streptokinase** gene **expression** in group A & C streptococci by two-component regulators
AUTHOR: Malke H (Reprint); Steiner K
CORPORATE SOURCE: Univ Jena, Inst Mol Biol, Winzerlaer Str 10, D-07745 Jena, Germany (Reprint); Univ Jena, Inst Mol Biol, D-07745 Jena, Germany
hmalke@imb-jena.de
COUNTRY OF AUTHOR: Germany
SOURCE: INDIAN JOURNAL OF MEDICAL RESEARCH, (MAY 2004) Vol. 119, Supp. [S], pp. 48-56.
ISSN: 0971-5916.
PUBLISHER: INDIAN COUNCIL MEDICAL RES, PO BOX 4911 ANSARI NAGAR, NEW DELHI 110029, INDIA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 23
ENTRY DATE: Entered STN: 13 Aug 2004
Last Updated on STN: 13 Aug 2004
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Background & objectives: Group A streptococci (GAS) and human isolates of group C streptococci (GCS) have the stable capacity to produce the plasminogen activator **streptokinase**, albeit with varying efficiency. This property is subject to control by two two-component regulatory systems, FasCAX and CovRS, which act as activator and repressor, respectively. The present work aims at balancing these opposing activities in GAS and GCS, and at clarifying the phylogenetic position of the FasA response regulator, the less understood regulator of the two systems.
Methods: The GCS strain H46A and GAS strain NZ131 were used. Escherichia **coli** JM 109 was used as host for plasmid construction. **Streptokinase** activity of various wild type and mutant strains was measured. Phylogenetic trees of streptococcal FasA

homologues were established.

Results: The **streptokinase** activities of the GAS strain NZ131 and the GCS strain H46A were attributable to more efficient CovR repressor action in NZ131 than in H46A. The FasA activator, on the other hand, functioned about equally efficient in the two strains. Phylogenetically, FasA homologues clustered distinctly in the proposed FasA-BlpR-ComE family of streptococcal response regulators and used the LytTR domain for DNA binding.

Interpretation & conclusion: Assessing the apparent **streptokinase** activity of streptoccal strains require the dissection of the activities of the cov and fas systems. Although experimental evidence is still missing, FasA is closely related to a widely distributed family of streptococcal response regulators that is involved in behavioral processes, such as quorum sensing.

L26 ANSWER 37 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:69727 SCISEARCH
THE GENUINE ARTICLE: 763KK
TITLE: **Streptokinase** - a clinically useful thrombolytic agent
AUTHOR: Banerjee A; Chisti Y; Banerjee U C (Reprint)
CORPORATE SOURCE: Natl Inst Pharmaceut Educ & Res, Dept Biotechnol, Sector 67, Mohali 160062, Punjab, India (Reprint); Natl Inst Pharmaceut Educ & Res, Dept Biotechnol, Mohali 160062, Punjab, India; Massey Univ, Inst Technol & Engn, Palmerston North, New Zealand
COUNTRY OF AUTHOR: India; New Zealand
SOURCE: BIOTECHNOLOGY ADVANCES, (FEB 2004) Vol. 22, No. 4, pp. 287-307.
ISSN: 0734-9750.
PUBLISHER: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND.
DOCUMENT TYPE: General Review; Journal
LANGUAGE: English
REFERENCE COUNT: 171
ENTRY DATE: Entered STN: 30 Jan 2004
Last Updated on STN: 30 Jan 2004

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A failure of hemostasis and consequent formation of blood clots in the circulatory system can produce severe outcomes such as stroke and myocardial infraction. Pathological development of blood clots requires clinical intervention with fibrinolytic agents such as urokinase, tissue plasminogen activator and **streptokinase**. This review deals with **streptokinase** as a clinically important and cost-effective plasminogen activator. The aspects discussed include: the mode of action; the structure and structure-function relationships; the structural modifications for improving functionality; **recombinant streptokinase**; microbial production; and recovery of this protein from crude broths. (C) 2003 Published by Elsevier Inc.

L26 ANSWER 38 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:148580 SCISEARCH
THE GENUINE ARTICLE: 285UW
TITLE: Allele substitution of the **streptokinase** gene reduces the nephritogenic capacity of group A streptococcal strain NZ131
AUTHOR: Nordstrand A (Reprint); McShan W M; Ferretti J J; Holm S E; Norgren M
CORPORATE SOURCE: Umea Univ, Dept Clin Bacteriol, S-90185 Umea, Sweden (Reprint); Univ Oklahoma, Hlth Sci Ctr, Dept Microbiol & Immunol, Oklahoma City, OK 73190 USA
COUNTRY OF AUTHOR: Sweden; USA

SOURCE: INFECTION AND IMMUNITY, (MAR 2000) Vol. 68, No. 3, pp. 1019-1025.
ISSN: 0019-9567.
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 36
ENTRY DATE: Entered STN: 2000
Last Updated on STN: 2000
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB To investigate the role of allelic variants of **streptokinase** in the pathogenesis of acute poststreptococcal glomerulonephritis (APSGN), site-specific integration plasmids were constructed, which contained either the non-nephritis-associated **streptokinase** gene (skc5) from the group C streptococcal strain Streptococcus **equisimilis** H46A or the nephritis-associated **streptokinase** gene (ska1) from the group A streptococcal nephritogenic strain NZ131. The plasmids were introduced by electroporation and homologous recombination into the chromosome of an isogenic derivative of strain NZ131, in which the **streptokinase** gene had been deleted and which had thereby lost its nephritogenic capacity in a mouse model of APSGN. The introduction of a non-nephritis-associated allelic variant of **streptokinase** did not rescue the nephritogenic capacity of the strain. The mutant and the wild-type strains produced equivalent amounts of **streptokinase**. Complementation of the ska deletion derivative with the original ska allele reconstituted the nephritogenicity of wild-type NZ131. The findings support the hypothesis that the role of **streptokinase** in the pathogenesis of APSGN is related to the allelic variant of the protein.

L26 ANSWER 39 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:100732 SCISEARCH
THE GENUINE ARTICLE: 277JH
TITLE: Genetic organisation of the M protein region in human isolates of group C and G streptococci: two types of multigene regulator-like (mgrC) regions
AUTHOR: Geyer A; Schmidt K H (Reprint)
CORPORATE SOURCE: Univ Jena, Univ Hosp, Inst Med Microbiol, Semmelweissstr 4, D-07740 Jena, Germany (Reprint); Univ Jena, Univ Hosp, Inst Med Microbiol, D-07740 Jena, Germany
COUNTRY OF AUTHOR: Germany
SOURCE: MOLECULAR AND GENERAL GENETICS, (JAN 2000) Vol. 262, No. 6, pp. 965-976.
ISSN: 0026-8925.
PUBLISHER: SPRINGER-VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 47
ENTRY DATE: Entered STN: 2000
Last Updated on STN: 2000
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In addition to beta-haemolytic streptococci belonging to Lancefield group A (Streptococcus pyogenes, GAS), human isolates of group C (GCS) and group G (GGS) streptococci (S. dysgalactiae subsp. **equisimilis**:) have been implicated as causative agents in outbreaks of purulent pharyngitis, of wound infections and recently also of streptococcal toxic shock-like syndrome. Very little is known about the organisation of the genomic region in which the emm gene of GCS and GCS is located. We have investigated the genome sequences flanking the emm gene in GCS by sequencing neighbouring fragments obtained by inverse PCR. Our sequence data for GCS strains 25287 and H46A revealed two types of arrangement in the emm region, which differ significantly from the known types of mga

regulon in GAS. We named this segment of the genome mgrC (for multigene regulon-like segment in group C streptococci). In strains belonging to the first mgrC type (prototype strain 25287) the emm gene is flanked upstream by mgc, a gene that is 61% identical to the mga gene of GAS. A phylogenetic analysis of the deduced protein sequences showed that Mgc is related to Mga proteins of various types of GAS but forms a distinct cluster. Downstream of emm, the mgrC sequence region is bordered by rel. This gene encodes a protein that functions in the synthesis and degradation of guanosine 3',5' bipyrophosphate (ppGpp) during the stringent regulatory response to amino acid deprivation. In the second mgrC type (prototype strain H46A), the genes mgc and emm are arranged as in type 1. But an additional ORF (orf) is inserted in opposite orientation between emm and rel. This orf shows sequence homology to cpdB, which is present in various microorganisms and encodes 2',3' cyclo-nucleotide 2'-phosphodiesterase. PCR analysis showed that these two mgrC arrangements also exist in GGS. Our sequence and PCR data further showed that both types of mgrC region in GCS and GGS are linked via rel to the **streptokinase** region characterised recently in strain H46A.
A gene encoding C5a peptidase, which is present at the 3' end of the mga regulon in GAS, was not found in the mgrC region identified in the GCS and GGS strains investigated here.

L26 ANSWER 40 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:666643 SCISEARCH
THE GENUINE ARTICLE: 231GM
TITLE: PauA: a novel plasminogen activator from Streptococcus uberis
AUTHOR: Rosey E L; Lincoln R A; Ward P N; Yancey R J; Leigh J A (Reprint)
CORPORATE SOURCE: Inst Anim Hlth, Compton Lab, Newbury RG20 7NN, Berks, England (Reprint); Pfizer Inc, Div Cent Res, Anim Hlth Biol Discovery, Groton, CT 06340 USA
COUNTRY OF AUTHOR: England; USA
SOURCE: FEMS MICROBIOLOGY LETTERS, (1 SEP 1999) Vol. 178, No. 1, pp. 27-33.
ISSN: 0378-1097.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 20
ENTRY DATE: Entered STN: 1999
Last Updated on STN: 1999

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Chromosomal DNA from two geographically distinct isolates of Streptococcus uberis was used to **clone** the plasminogen activator in an active form in Escherichia **coli**. The **cloned** fragments from each strain contained four potential open reading frames (ORFs). That for the plasminogen activator encoded a protein of 286 amino acids (33.4 kDa) which is cleaved between residues 25 and 26 during secretion by S. uberis. The amino acid sequence of the mature protein showed only weak homology (23.5-28%) to **streptokinase**. The plasminogen activator gene, pauA, in S. uberis was located between two ORFs with high homology to the DNA mismatch repair genes, hexA and hexB, and not on a DNA fragment between the genes encoding an ATP binding cassette transporter protein (abc) and a protein involved in the formation and degradation of guanosine polyphosphates (rel) as is the case for **streptokinase** in other streptococci. (C) 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V.

L26 ANSWER 41 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:317401 SCISEARCH
THE GENUINE ARTICLE: 189BZ
TITLE: Hydrophobic interaction chromatography applied to purification of **recombinant streptokinase**
AUTHOR: Perez N (Reprint); Urrutia E; Camino J; Orta D R; Torres Y; Martinez Y; Cruz M; Alburquerque S; Gil M R; Hernandez L
CORPORATE SOURCE: Ctr Genet Engn & Biotechnol, Streptokinase Div, Havana, Cuba; Ctr Genet Engn & Biotechnol, Qual Control Div, Havana, Cuba
COUNTRY OF AUTHOR: Cuba
SOURCE: MINERVA BIOTECNOLOGICA, (DEC 1998) Vol. 10, No. 4, pp. 174-177.
ISSN: 1120-4826.
PUBLISHER: EDIZIONI MINERVA MEDICA, CORSO BRAMANTE 83-85 INT JOURNALS DEPT., 10126 TURIN, ITALY.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 22
ENTRY DATE: Entered STN: 1999
Last Updated on STN: 1999
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background **Recombinant streptokinase** (rSk) is a streptococcal protein **cloned** in E. **coli**.(11) Several methods have been described for **streptokinase** purification: ion exchange chromatography,(12) affinity chromatography with canine plasmin(13) and chromatography on immobilized acylated human plasminogen.(14) Monoclonal antibodies anti-rSk immobilized to Sepharose(15) have been used too. Recently this protein was purified using HIC.

Methods. rSk (CIGB, Cuba) was produced by fermentation strain K12 of E. **coli**,(11) the protein was recovered after washed pellet, cellular disruption and solubilization. Several purification assays were done using TSK-Butyl (Tosohaas, Japan) as a support for hydrophobic interaction chromatography (HIC). The protein was loaded to the column with 1 M of ammonium sulfate before being washed using an elution gradient from 0.5 to 0 M of ammonium sulfate, in order to determine the elution point of the rSk.

Results. Wie could determine that this protein is partly hydrophobic, this determination was shown by analysis of its aminoacidic sequence. This protein has 415 aminoacids of which 36% are non polar. The absorption capacity for TSK Butyl 650 S varies from 15 to 20 mg/mL. The optimum elution point was obtained using 0.25 M of ammonium sulfate, the eluted material was obtained with a high level of purity (<1% of contaminants). The recovery of rSk was about 49% using the mean of five assays.

Conclusions. The experimental process evaluated could be efficiently inserted in a downstream process to obtain **recombinant streptokinase** highly purified as final preparation and good recovery.

L26 ANSWER 42 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1998:473042 SCISEARCH
THE GENUINE ARTICLE: ZV077
TITLE: The interaction of Streptococcus dysgalactiae with plasmin and plasminogen
AUTHOR: Leigh J A (Reprint); Hodgkinson S M; Lincoln R A
CORPORATE SOURCE: Inst Anim Hlth, Compton Lab, Newbury RG20 7NN, Berks, England (Reprint)
COUNTRY OF AUTHOR: England
SOURCE: VETERINARY MICROBIOLOGY, (15 MAR 1998) Vol. 61, No. 1-2, pp. 121-135.

ISSN: 0378-1135.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 29
ENTRY DATE: Entered STN: 1998
Last Updated on STN: 1998
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The activation of plasminogen and the binding of plasmin by bacteria may have many effects which promote infection. The occurrence of such activities in streptococci is well documented; however, these are yet to be demonstrated for S. dysgalactiae. Consequently, the ability of this bacterium to activate mammalian plasminogen and bind either plasmin or its zymogen was investigated. Activation of bovine plasminogen was dependant on both the strain and the growth medium used for cultivation. Eighteen strains were able to activate bovine and ovine plasminogen and some of these also activated plasminogen from the horse, rabbit and pig. None activated human plasminogen and one strain (CE127) did not activate plasminogen from any source. Tricine-SDS PAGE and zymographic analysis of culture supernatants showed that bovine plasminogen was activated by four out of six strains at two locations corresponding to 16 kDa and IO kDa. Following the growth of five strains in the presence of bovine plasminogen, all but strain CE127 bound high levels of plasmin activity. In contrast, following growth in human plasminogen none of the strains exhibited bound plasmin activity although all could bind human plasmin directly. Ail strains were also able to bind bovine and human plasminogen in such a way as to allow its activation by urokinase. We conclude that S. dysgalactiae is capable of activating mammalian plasminogen in a species-specific fashion and that the bacterium is also capable of binding plasmin and plasminogen with an apparent preference for bovine plasmin over human plasmin and/or plasminogen from either species. (C) 1998 Elsevier Science B.V.

L26 ANSWER 43 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1998:437021 SCISEARCH
THE GENUINE ARTICLE: ZU066
TITLE: The Streptococcus agalactiae hylB gene encoding hyaluronate lyase: completion of the sequence and **expression** analysis
AUTHOR: Gase K; Ozegowski J; Malke H (Reprint)
CORPORATE SOURCE: Univ Jena, Inst Mol Biol, Winzerlaer Str 10, D-07745 Jena, Germany (Reprint); Univ Jena, Inst Mol Biol, D-07745 Jena, Germany; Univ Jena, Inst Expt Microbiol, D-07745 Jena, Germany
COUNTRY OF AUTHOR: Germany
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA-GENE STRUCTURE AND EXPRESSION, (29 MAY 1998) Vol. 1398, No. 1, pp. 86-98. ISSN: 0167-4781.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 43
ENTRY DATE: Entered STN: 1998
Last Updated on STN: 1998
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We report the **cloning**, sequencing and **expression** analysis of the Streptococcus agalactiae strain 4755 hylB(4755) allele, the first chromosomally-encoded streptococcal hyaluronate lyase gene to be **cloned** and sequenced completely. This gene lies in a region homologous to that found in S. mutans, between the mutX and rmlB genes, a region involved in the synthesis of the serotype c-specific polysaccharide

antigen of this organism. Sequencing of hylB(4755) revealed a 3216-bp open reading frame that encodes a 121.2-kDa polypeptide possessing a 30-amino acid signal sequence which was theoretically predicted and experimentally confirmed. A **recombinant** plasmid, pHYB100, containing hylB(4755) together with its promoter and terminator was constructed and used to analyze the **expression** of the gene in Escherichia **coli**. In Northern hybridization experiments, hylB(4755) was found to be transcribed as 3.3-kb monocistronic mRNA from its own promoter which exhibits an extended, sigma(70)-like 10 consensus sequence. Transcript mapping by primer extension analysis placed the major transcription initiation site leading to the longest transcript 38 bp upstream of the translational initiation codon: ATG. E. **coli** TG1(pHYB100) efficiently synthesized hyaluronan-cleaving enzyme activity at similar to 7000 working units/10(9) cells, with lyase activity detectable in all principle cellular locations. Zymography and Western analysis identified functional activity in TG1(pHYB100) to be associated with similar to 118, 110 and 94-kDa polypeptides, with the two low molecular weight species constituting the major components of the enzyme purified from the culture supernatant fluid of S. agalactiae 4755. The 118-kDa form was shown to represent the undegraded mature enzyme, whereas the smaller species are likely to arise from proteolytic cleavage in the N-terminal part of the mature protein. The HylB(4755) protein showed extensive sequence identity to the homologous enzymes from S. agalactiae 3502 and S. pneumoniae characterized by others but sequence comparisons clearly show that incomplete genes truncated at their 5' ends had been isolated from these two organisms.

L26 ANSWER 44 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1997:547160 SCISEARCH
THE GENUINE ARTICLE: XL484
TITLE: The LppC gene of Streptococcus **equisimilis** encodes a lipoprotein that is homologous to the e(P4) outer membrane protein from Haemophilus influenzae
AUTHOR: Gase K (Reprint); Liu G W; Bruckmann A; Steiner K; Ozegowski J; Malke H
CORPORATE SOURCE: UNIV JENA, INST MOL BIOL, D-07745 JENA, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: MEDICAL MICROBIOLOGY AND IMMUNOLOGY, (JUN 1997) Vol. 186, No. 1, pp. 63-73.
ISSN: 0300-8584.
PUBLISHER: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 39
ENTRY DATE: Entered STN: 1997
Last Updated on STN: 1997
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We report the **cloning**, sequencing, and analysis of a novel chromosomal gene of Streptococcus **equisimilis** strain H46A that codes for a membrane lipoprotein, designated LppC. The lppC gene is located 3' adjacent to, and co-oriented with, the unrelated gapC gene that encodes the previously characterized glyceraldehyde-3-phosphate dehydrogenase. Sequencing of lppC revealed an 855-bp open reading frame that predicted a 32.4-kDa polypeptide possessing a potential lipoprotein signal sequence and modification site (VTGC). Signal sequence processing of LppC synthesized in the homologous host or **expressed** from plasmid pLPP2 in Escherichia **coli** was sensitive to globomycin, a selective inhibitor of lipoprotein-specific signal peptidase II. Subcellular localization of LppC using polyclonal antibodies raised to the hexahistidyl-tagged protein proved LppC to be tightly associated with the cytoplasmic membrane of S. **equisimilis** and with the outer

membrane of E. **coli** JM109 (pLPP2). Southern, Northern and Western analyses indicated that Ipl, was conserved in S. pyogenes, and transcribed independently of gap as monocistronic 0.9-kb mRNA from a sigma(70)-like consensus promoter. Database searches found homology of LppC to the hel gene-encoded outer membrane protein e (P4) from Haemophilus influenzae to which it exhibits 58% sequence similarity. However, unlike the hel gene, lppC was unable to complement hemA mutants of E. **coli** for growth on hemin as sole porphyrin source in aerobic conditions. Furthermore, neither the wild type nor an lppC insertion mutant of S. **equisimilis** could grow on hemin in iron-limited medium. These results, together with findings indicating that S. **equisimilis** H46A had no absolute requirement for iron, led us to conclude that lppC, in contrast to hel, is not involved in hemin utilization and has yet to be assigned a function.

L26 ANSWER 45 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1996:43901 SCISEARCH
THE GENUINE ARTICLE: TN446
TITLE: **Cloning** and sequencing of the **streptokinase** gene from Streptococcus pyogenes (CIP 56.57)
AUTHOR: Ball M M (Reprint); Puig J; Iborra F
CORPORATE SOURCE: UNIV PARIS 11, F-91405 ORSAY, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: DNA SEQUENCE, (1995) Vol. 6, No. 1, pp. 33-36.
ISSN: 1042-5179.
PUBLISHER: HARWOOD ACAD PUBL GMBH, C/O STBS LTD, PO BOX 90, READING, BERKS, ENGLAND RG1 8JL.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 10
ENTRY DATE: Entered STN: 1996
Last Updated on STN: 1996
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The **streptokinase** gene of the Streptococcus pyogenes strain CIP 56.57 was **cloned** and sequenced. This sequence coding for a 441 amino acid protein is well conserved among streptococcus species: there are two very conserved domains separated by a more variable region.

L26 ANSWER 46 OF 71 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1995:622543 SCISEARCH
THE GENUINE ARTICLE: RU780
TITLE: **STREPTOKINASE**-MEDIATED PLASMINOGEN ACTIVATION USING A **RECOMBINANT** DUAL FUSION PROTEIN CONSTRUCT - A NOVEL-APPROACH TO STUDY BACTERIAL HOST PROTEIN INTERACTIONS
AUTHOR: LIZANO S (Reprint); JOHNSTON K H
CORPORATE SOURCE: LOUISIANA STATE UNIV, MED CTR, DEPT MICROBIOL IMMUNOL & PARASITOL, NEW ORLEANS, LA 70112
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF MICROBIOLOGICAL METHODS, (SEP 1995) Vol. 23, No. 3, pp. 261-280.
ISSN: 0167-7012.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 59
ENTRY DATE: Entered STN: 1995
Last Updated on STN: 1995

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Streptokinase** (SK), a plasminogen (Pg) activator secreted by groups A, C, and G streptococci, is extensively used as a pharmacological agent in thrombolytic therapy and possibly plays a role in streptococcal invasiveness and disease. SK activates Pg to plasmin (Ps) by forming an activator complex with Pg. However, the molecular basis whereby SK binds and activates Pg remains unclear, in part due to the rapid fragmentation of the SK-Pg complex. This study describes a solid phase approach to study this interaction in which a **recombinant** SK molecule was constructed with glutathione-S-transferase appended to the NH2 terminus and (Gly)(3)(His)(8) appended to the COOH terminus. This dual fusion protein molecule, immobilized on either Sepharose-S-hexylglutathione or Ni2+ dinitriloacetic acid-Sepharose was then used to study the interaction of SK with Pg. These SK-Pg complexes exhibited amidolytic and proteolytic activity similar to native SK, but the pattern of fragmentation of the SK molecule was dependent upon whether the SK molecule was immobilized either at its NH2- or COOH terminus. This solid phase approach may contribute to a greater understanding of the role of SK in Pg activation by enabling the 'capture' of intact activator complexes under physiological conditions and, in addition, may serve as a useful model to analyze other bacterial-host protein interactions important in the pathogenesis of disease.

L26 ANSWER 47 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2005:29440 HCAPLUS
DOCUMENT NUMBER: 142:428913
TITLE: An improved process for the simultaneous preparation of extracellular **streptokinase** and its analogues
INVENTOR(S): Dikshit, Kanak Lata; Vyas, Vinay Venkatrao; Mahajan, Ritu; Kaur, Jaodeep; Thapar, Nitika; Phatap, Jitesh; Nihalani, Deepak; Sahni, Girish
PATENT ASSIGNEE(S): Council of Scientific and Industrial Research, India
SOURCE: Indian, 122 pp.
CODEN: INXXAP
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
IN 183828	A	20000429	IN 1994-DE1727	19941230

PRIORITY APPLN. INFO.: IN 1994-DE1727 19941230

AB Extracellular **streptokinase** and its analogs prepared by growing **recombinant** E.**coli** in a conventional fermentation medium under stirring and supplemented with aeration, separating the cells from supernatant by known methods followed by recovering and purifying **Streptokinase** and its analogs from supernatant.

L26 ANSWER 48 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:175570 HCAPLUS
DOCUMENT NUMBER: 132:218864
TITLE: **Streptokinase** analogs with low antigenicity for use as thrombolytics
INVENTOR(S): Torrens Madrazo, Isis Del Carmen; Garcia Ojalvo, Ariana; De La Fuente Garcia, Jose De Jesus; Seralena Menendez, Alina
PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba
SOURCE: Eur. Pat. Appl., 54 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 985729	A2	20000315	EP 1999-202639	19990813
EP 985729	A3	20000531		
EP 985729	B1	20050427		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
AU 9943424	A1	20000309	AU 1999-43424	19990805
AU 769916	B2	20040212		
CA 2277554	C	20041102	CA 1999-2277554	19990806
CA 2277554	AA	20000214		
US 6309873	B1	20011030	US 1999-374038	19990813
AT 294239	E	20050515	AT 1999-202639	19990813
US 6413759	B1	20020702	US 2000-658179	20000908
PRIORITY APPLN. INFO.:			CU 1998-119 A	19980814
			US 1999-374038 A3	19990813

AB **Streptokinase** analogs with antigenic domains modified to minimize antigenicity are described for use in the treatment of clotting-associated disorders. The proteins retain their capacity for plasminogen activátor complex formation. The proteins are manufactured by **expression** of the corresponding allele of the skc2 gene encoding **streptokinase SKC-2** (Heberkinase®). The mols. obtained from present invention can be used in the treatment of disorders as myocardial infarction, pulmonary thromboembolism, surgical complications and other cases of thrombosis.

L26 ANSWER 49 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1999:579938 HCAPLUS
DOCUMENT NUMBER: 131:183948
TITLE: Manufacture of **streptokinase** for therapeutic use by **expression** of the **cloned** gene in Pichia pastoris
INVENTOR(S): Estrada Garcia, Mario; Rubiera Chaplen, Roger; Perez, Hidalgo; Serrano Doce, Ricardo; Hernandez Marrero, Luciano F.; Rodriguez Collazo, Pedro; Castro Ramirez, Anaisel; Munoz Munoz, Emilio Amable; Bravo Martinez, Walfrido; Campos Somavilla, Magalys; Pedraza Fernandez, Alicia; De la Furente Garcia, Jose de J.; Herrera Martinez, Luis S.
PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba
SOURCE: Czech Rep., 18 pp.
CODEN: CZXXED
DOCUMENT TYPE: Patent
LANGUAGE: Czech
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CZ 284692	B6	19990217	CZ 1991-2256	19910719
RU 2107726	C1	19980327	RU 1991-5001280	19910717
PRIORITY APPLN. INFO.:			CU 1990-90 A	19900523
			CS 1991-2256 A	19910719
			SU 1991-5001280 A	19910717

AB **Streptokinase** for therapeutic use is manufactured by **expression** of the **cloned** gene in Pichia pastoris. The protein may be secreted into the culture medium or accumulated intracellularly. The gene was **cloned** from a type C Streptococcus **equisimilis** by PCR. **Expression** of the gene from the AOX1 promoter using Pichia pastoris as the host resulted in

the manufacture of an enzyme with a specific activity of 50,000-100,000 fibrin-agarose units/mg protein.

L26 ANSWER 50 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1999:405093 HCAPLUS
DOCUMENT NUMBER: 131:54027
TITLE: Fibrin-dependent plasminogen activator activity of modified bacterial **streptokinases**
INVENTOR(S): Reed, Guy L.
PATENT ASSIGNEE(S): The President and Fellows of Harvard College, USA
SOURCE: PCT Int. Appl., 73 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 9931247	A1	19990624	WO 1998-US26694		19981215
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM					
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG					
AU 9918295	A1	19990705	AU 1999-18295		19981215
US 6210667	B1	20010403	US 1998-211542		19981215
PRIORITY APPLN. INFO.:			US 1997-69497P	P	19971215
			WO 1998-US26694	W	19981215

AB A pharmaceutical composition in a preferred embodiment comprises an isolated bacterial protein **streptokinase** that induces fibrin-dependent plasminogen activation, and methods for dissolving blood clots in a subject using such a composition Two preferred **streptokinase** mutants and truncated derivs. comprising residues 144-293 and residues 60-414 of the Streptococcus **equisimilis** H46A enzyme. Deletion of the first 59 amino acids to product mutant rSK60-414 yielded a protein with a 767-fold decrease in kcat compared to that of rSK1-414, without any significant change in the Km. The N-terminus dets. the clot (fibrin) dependence of plasminogen activation by **streptokinase** and the regulation of plasminogen activation in the presence of fibrin. By virtue of its requirement for fibrin for plasminogen activation in human plasma, and its sparing of fibrinogen during clot dissoln., **streptokinase** deleted of N-terminal amino acid residues is similar to tissue-type plasminogen activator. Embodiments also include a nucleic acid encoding such as a bacterial protein, a nucleic acid encoding such a bacterial protein as a fusion to another protein, an **expression** vector with the nucleic acid, and a host cell transformed with the **expression** vector.
REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 51 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1997:155090 HCAPLUS
DOCUMENT NUMBER: 126:154444
TITLE: **Streptokinases** analogs resistant to cleavage and inactivation by plasmin
INVENTOR(S): Reed, Guy L.
PATENT ASSIGNEE(S): President and Fellows of Harvard College, USA
SOURCE: PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent

LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9641883	A1	19961227	WO 1996-US9640	19960607
W: CA, JP, MX				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5854049	A	19981229	US 1995-488940	19950609
PRIORITY APPLN. INFO.:			US 1995-488940 A	19950609

AB **Streptokinase** analogs with altered plasmin-binding features that are resistant to binding by plasmin and subsequent cleavage and inactivation are described for use as thrombolytics with a prolonged serum half-life. Specifically, analogs of the Streptococcus **equisimilis** **streptokinase** are described. Changes that increase plasminogen resistance include alterations of the plasmin-binding domain and and blocking of the N-terminus. Fusion proteins with maltose-binding protein as the N-terminal moiety are prepared and their plasmin resistance and **streptokinase** activity are described. Similarly, analogs with substitutions of basic amino acids that identify internal plasmin and trypsin cleavage sites were prepared and characterized.

L26 ANSWER 52 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1993:229181 HCAPLUS
DOCUMENT NUMBER: 118:229181
TITLE: **Cloning** and **expression** of a gene for **streptokinase** from a hemolytic Streptococcus
INVENTOR(S): Garcia, Mario P. E.; Chaplen, Roger R.; Hidalgo, Aimee P.; Doce, Ricardo S.; Marrero, Luciano F. H.; Collazo, Pedro R.; Ramirez, Anaisel C.; Munoz, Emilio A. M.; Martinez, Walfrido B.; et al.
PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba
SOURCE: Can. Pat. Appl., 26 pp.
CODEN: CPXXEB
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CA 2043953	AA	19921206	CA 1991-2043953	19910605
CA 2043953	C	20010612		
HU 62655	A2	19930528	HU 1991-1770	19910527
HU 216073	B	19990428		
SK 279873	B6	19990507	SK 1991-2256	19910719
PRIORITY APPLN. INFO.:			CA 1991-2043953	19910605

AB The gene for **streptokinase** of a Streptococcus **equisimilis** type C is **cloned** and **expressed** in Escherichia **coli** or in yeasts. **Expression** in yeasts uses the promoter of the AOX1 gene of Pichia pastoris to regulate **expression**. Secretion of the protein was achieved using the cognate signal peptide or one from sucrose invertase. The gene was **cloned** by PCR amplification using different pairs of primers to **clone** the gene with or without the signal sequence. Integrating **expression** vectors for **expression** of the gene in Pichia pastoris with or without secretion of the product were constructed. When the secretory construct was used, **streptokinase** yields of 1-1.2 g/L were obtained. The protein had the expected biol. activities, and the purified enzyme had a specific activity of 50,000-100,000 units/mg.

L26 ANSWER 53 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1992:646505 HCAPLUS
DOCUMENT NUMBER: 117:246505
TITLE: **Streptokinase** mutation affecting **skc** **expression** in homologous and heterologous hosts
AUTHOR(S): Mechold, U.; Muller, J.; Malke, H.
CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Ther., Jena, D-6900, Germany
SOURCE: Zentralblatt fuer Bakteriologie, Supplement (1992), 22(New Perspect. Streptococci Streptococcal Infect.), 336-8
CODEN: ZBASE2; ISSN: 0941-018X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Mutations affecting the level of **streptokinase** gene **skc** **expression** and/or secretion in homologous and heterologous hosts are phys. characterized. The principal classes of mutations produced included **skc** deletions, IS element insertions, and **skc** duplications. The deletion events, represented by mutations Δ(**skc**)-247 and Δ(**skc**)-305 present in plasmids pMM247 and pMM305, resp., removed a tetrapeptide (F10-L13 or L12-A15) from the hydrophobic core of the **Skc** signal sequence. These mutations, reduced the size, hydrophobicity and predicted alpha-helicity of the central region of the signal sequence. The corresponding plasmids, upon transformation into E. **coli** and P. mirabilis L-forms, substantially increased the level of **Skc** **expression** in either host. In E. **coli**, they also facilitated the export of mature **Skc** into the culture medium. In the gram-pos. hosts, **skc** **expression** was less dramatically affected; however, the proportion of **Skc** activity found in the culture medium was significantly decreased when compared to the extracellular activity resulting from wild type **skc**. IS1 insertion did not alter the primary structure of the promoter but displaced in upward direction, by 768 bp, a static DNA bending locus having its center some 140 bp upstream of the -35 region in wild type DNA. When studied with plasmid pMM697, this insertion event resulted in severely decreased **Skc** **expression** in all hosts but, expectedly, did not affect **Skc** secretability. Gene **skc** duplication in the chromosome of the homologous producer strain, S. **equisimilis** H46A, was achieved by a single crossover event between the chromosomes and an integrateable **Skc** plasmid, pSM752, in the region of shared homol. As judged by Southern hybridization, cells transiently supporting the replication of pSM752 gave rise to a stable erythromycin-resistant **clone** designated H46SM which was plasmid-free and produced **Skc** at levels approx. twice as high as the wild type.

L26 ANSWER 54 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1992:585755 HCAPLUS
DOCUMENT NUMBER: 117:185755
TITLE: High-level **expression** of degraded product-free **streptokinase** in Escherichia **coli** by removal of its putative leader sequence. [Erratum to document cited in CA116(13):122160m]
AUTHOR(S): Park, Seung Kook; Jang, Jeong Su; Kim, Jee Cheon; Chun, Moon Jin; Byun, Si Myung
CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol., Seoul, 130-650, S. Korea
SOURCE: Molecules and Cells (1992), 2(1), 119
CODEN: MOCEEK; ISSN: 1016-8478
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The omission of acknowledgment of a research grant has been corrected The

error was not reflected in the abstract or the index entries.

L26 ANSWER 55 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1992:441935 HCAPLUS
DOCUMENT NUMBER: 117:41935
TITLE: **Cloning** and **expression** of **streptokinase** gene of C-type Streptococcus **equisimilis**
PATENT ASSIGNEE(S): Centro de Ingenieria Genetica y Biotecnologia (CIGB), Cuba
SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 04030794	A2	19920203	JP 1990-201600	19900731
JP 3127298	B2	20010122		
EP 489201	A1	19920610	EP 1990-201930	19900717
EP 489201	B1	19951115		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 130369	E	19951215	AT 1990-201930	19900717
ES 2081909	T3	19960316	ES 1990-201930	19900717
US 5296366	A	19940322	US 1991-703778	19910522
AU 644657	B2	19931216	AU 1991-78101	19910531
RU 2107726	C1	19980327	RU 1991-5001280	19910717
PRIORITY APPLN. INFO.:			CU 1990-90 A	19900523
			SU 1991-5001280 A	19910717

AB The **streptokinase** (I) gene **SKC-2** ,with/without signal sequence, is **cloned** from C-type S. **equisimilis** ATCC-9542 by the polymerase chain reaction method and **expressed** in Escherichia **coli** and yeast for com. manufacture of I. Genomic DNA of the C-type S. **equisimilis** was isolated by the standard method and amplified with primers derived from the nucleotide sequence of **SKC** to get I gene with/without signal sequence. **Expression** of the I gene in E. **coli** and Pichia pastoris MP-36 mutant were shown. The production of I with these microorganisms were ≥350 mg/L and ≥1.2 g/L, resp.

L26 ANSWER 56 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1992:122160 HCAPLUS
DOCUMENT NUMBER: 116:122160
TITLE: High-level **expression** of degraded product-free **streptokinase** in Escherichia **coli** by removal of its putative leader sequence
AUTHOR(S): Park, Seung Kook; Jang, Jeong Su; Kim, Jee Cheon; Chun, Moon Jin; Byun, Si Myung
CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol., Seoul, 130-650, S. Korea
SOURCE: Molecules and Cells (1991), 1(2), 187-92
CODEN: MOCEEK; ISSN: 1016-8478
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **expression** vector for **streptokinase** has been constructed from the previously **cloned streptokinase** -coding gene (**skc**) from Streptococcus **equisimilis**. Because of its deleterious effect on the host cell growth, the leader sequence of **skc** was removed and the leader sequence-deleted **skc** was subcloned into the vector pKK223-3, which contains the regulatable tac promoter and rrnB T1T2 transcription terminator, with a

short synthetic oligonucleotide adapter. When this vector, pKS601 having **skc** gene, was **expressed** in E. **coli**, a 47.4-kDa protein was found to be newly accumulated to about 12% of the total cellular proteins, and it was identified as the **streptokinase** by immunoblotting with rabbit anti-**streptokinase** polyclonal serum. The **expressed streptokinase** was free from carboxyl-terminal degraded 44-kDa **streptokinase** and purified to near homogeneity using DEAE-cellulose and Sephadex G-150 columns. Its specific activity was about 1.3 + 105 CLN units/mg protein.

L26 ANSWER 57 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:672660 HCAPLUS
DOCUMENT NUMBER: 115:272660
TITLE: **Recombinant** of thrombolytic and fibrinolytic enzymes as inactive dimers linked by sequence recognized by blood coagluation factors
INVENTOR(S): Dawson, Keith Martyn; Hunter, Michael George; Czaplewski, Lloyd George
PATENT ASSIGNEE(S): British Bio-Technology Ltd., UK
SOURCE: PCT Int. Appl., 110 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 9109125	A1	19910627	WO 1990-GB1911		19901207
W: AU, CA, FI, HU, JP, KR, NO, US					
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE					
CA 2069085	AA	19910608	CA 1990-2069085		19901207
CA 2069085	C	20000201			
CA 2069105	AA	19910608	CA 1990-2069105		19901207
AU 9169540	A1	19910718	AU 1991-69540		19901207
AU 644399	B2	19931209			
ZA 9009853	A	19920826	ZA 1990-9853		19901207
ZA 9009854	A	19920826	ZA 1990-9854		19901207
EP 504241	A1	19920923	EP 1991-900869		19901207
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE					
AT 155812	E	19970815	AT 1991-900851		19901207
ES 2106073	T3	19971101	ES 1991-900851		19901207
IL 96601	A1	19990509	IL 1990-96601		19901207
JP 05502374	T2	19930428	JP 1991-501314		19911115
JP 2900606	B2	19990602			
US 5434073	A	19950718	US 1992-854596		19920603
FI 9202609	A	19920605	FI 1992-2609		19920605
NO 9202237	A	19920806	NO 1992-2237		19920605
AU 9344976	A1	19931118	AU 1993-44976		19930830
PRIORITY APPLN. INFO.:			GB 1989-27722	A	19891207
			WO 1990-GB1911	A	19901207

AB Fibrinolytic or thrombolytic enzymes are manufactured in a **recombinant** host as inactive fusion proteins containing two or more sequences of the protein linked by a peptide that can be cleaved by a blood-coagulation factor. The construction of **expression** vectors for the manufacture of hirudin or **streptokinase** dimers linked by peptides cleavable by Factor Xa or thrombin for Escherichia col or Saccharomyces cerevisiae (with or without product secretion) is described. All the products tested were cleavable by the appropriate factors.

L26 ANSWER 58 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:443480 HCAPLUS
DOCUMENT NUMBER: 115:43480
TITLE: Synthetic genes for **streptokinase** and

streptokinase analogs and their **expression** in Escherichia **coli**

INVENTOR(S): Fujii, Setsuro; Katano, Tamiki; Majima, Eiji; Ogino, Koichi; Ono, Kenji; Sakata, Yasuyo; Uenoyama, Tsutomu

PATENT ASSIGNEE(S): Otsuka Pharmaceutical Factory, Inc., Japan

SOURCE: Eur. Pat. Appl., 76 pp.
CODEN: EPXXDW

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
EP 407942	A2	19910116	EP 1990-113099		19900709
EP 407942	A3	19910904			
EP 407942	B1	19951011			
R: AT, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE					
JP 04011892	A2	19920116	JP 1990-179851		19900706
US 5240845	A	19930831	US 1990-549049		19900706
AU 9058806	A1	19910117	AU 1990-58806		19900709
AU 648029	B2	19940414			
AT 129014	E	19951015	AT 1990-113099		19900709
ES 2078925	T3	19960101	ES 1990-113099		19900709
CA 2020828	AA	19910112	CA 1990-2020828		19900710
PRIORITY APPLN. INFO.:			JP 1989-179432	A	19890711
			JP 1989-307957	A	19891127
			JP 1990-96830	A	19900411

AB Genes encoding **streptokinase** (I) and its derivs. are synthesized and **expressed** in a host such as Escherichia **coli** for manufacture of I suitable for clin. application. The DNA encoding natural-type I was synthesized by standard chemical and used for construction of **expression** plasmid pSKXT, which in turn **expressed** the I gene using the E. **coli** tac promoter and the blc signal sequence. Efficient **expression** of the gene in the E. **coli** transformants and purification of the protein product were demonstrated. I analogs with a carboxy-terminal deletions, optionally with internal modifications were also described.

L26 ANSWER 59 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1991:179754 HCAPLUS

DOCUMENT NUMBER: 114:179754

TITLE: Fusion proteins of **streptokinase** and human plasminogen

INVENTOR(S): Malke, Horst; Ferretti, Joseph J.

PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Zentralinstitut fuer Mikrobiologie und Experimentelle Therapie, Ger. Dem. Rep.

SOURCE: Ger. (East), 42 pp.
CODEN: GEXXA8

DOCUMENT TYPE: Patent

LANGUAGE: German

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 284484	A5	19901114	DD 1989-328631	19890516
PRIORITY APPLN. INFO.:			DD 1989-328631	19890516

AB A hybrid **streptokinase** is produced in prokaryotic cells. The **streptokinase**, which displays thrombin selectivity, consists of the N-terminal kringle domains of human plasminogen fused to C-terminal Streptococcus **equisimilis streptokinase**. Escherichia **coli** transformed with plasmids encoding the described fusion

protein fused to the N-terminal hexapeptide of β-galactosidase produced the hybrid **streptokinase** which was purified from cell lysates by immuno-affinity chromatog. and by chromatog. on lysine Sepharose.

L26 ANSWER 60 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:162447 HCAPLUS
DOCUMENT NUMBER: 114:162447
TITLE: **Recombinant** manufacture of **streptokinase**
INVENTOR(S): Laplace, Frank; Mueller, Joerg; Malke, Horst
PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Patentabteilung, Ger. Dem. Rep.
SOURCE: Ger. (East), 9 pp.
CODEN: GEXXA8
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 282709	A5	19900919	DD 1988-323948	19881227
PRIORITY APPLN. INFO.:			DD 1988-323948	19881227

AB **Streptokinase** from Streptococcus **equisimilis** serotype C is manufactured by **expression** of the **skc** gene in Escherichia **coli**, Bacillus subtilis, or other Streptococcus. The natural **expression** cassette for the **skc** gene was introduced into a broad host-range vector to give plasmid pMLS10. Transformants of Streptococcus sanguis carrying this vector produced 750-1,000 **streptokinase** units/mL in a complex medium after 16 h growth at 36°, at this point the culture reached stationary phase and the enzyme continued to be slowly accumulated.

L26 ANSWER 61 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:96194 HCAPLUS
DOCUMENT NUMBER: 114:96194
TITLE: The leader sequence of **streptokinase** is responsible for its post-translational carboxyl-terminal cleavage
AUTHOR(S): Park, Seung Kook; Lee, Byeong Ryong; Byun, Si Myung
CORPORATE SOURCE: Dep. Biol. Sci. Eng., Korea Adv. Inst. Sci. Technol., Seoul, 130-650, S. Korea
SOURCE: Biochemical and Biophysical Research Communications (1991), 174(1), 282-6
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB When the **streptokinase** gene from Streptococcus equisimillis was **expressed** from 2 tac promoter-controlled **expression** vectors, one deleted the putative leader sequence of **streptokinase**. Both normal and degraded **streptokinase** were detected in proteins **expressed** from the leader-encoding vector, but only normal **streptokinase** was detected from the leader-deleted vector. These findings indicate that the characteristic carboxyl-terminal cleavage of **streptokinase** is correlated with its leader sequence and occurs during defective secretion. A homogeneous preparation of **streptokinase** was facilitated by **expression** from this leader-deleted vector.

L26 ANSWER 62 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1989:206803 HCAPLUS
DOCUMENT NUMBER: 110:206803
TITLE: **Streptokinase** mutations relieving

Escherichia **coli** K-12 (prlA4) of detriments caused by the wild-type **skc** gene
AUTHOR(S): Mueller, Joerg; Reinert, Hilmer; Malke, Horst
CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Therapy, Acad. Sci. G. D. R., Jena, 6900, Ger. Dem. Rep.
SOURCE: Journal of Bacteriology (1989), 171(4), 2202-8
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A novel phenotype is described for E. **coli** K-12 carrying the prlA4 allele determining a membrane component of the protein export mechanism. It is manifest as transformation deficiency for plasmids containing the **cloned** group C streptococcal **streptokinase** gene, **skc**. **Streptokinase** plasmid mutations relieving the prlA4 strain of this deficiency fell into three classes. Class 1 included **skc**::IS5 insertions, with IS5 integrated in a region encoding the **Skc** signal sequence and inactivating **skc**. Class 2 included IS1 insertions leaving **skc** intact but reducing **skc expression**, presumably by altering the function of the **skc** promoter as judged by an insertion site close to the -35 region. Class 3 included **skc** deletions removing the entire signal sequence or a tetrapeptide from its hydrophobic core. The tetrapeptide deletion reduced the size, hydrophobicity, and predicted α-helicity of the central region of the **Skc** signal sequence but facilitated the export of mature **Skc** in both the wild type and the prlA4 mutant. These findings indicate that the incompatibility between prlA4 and **skc** is related to deleterious effects of the **Skc** signal sequence. The tetrapeptide deletion may function by altering the conformation of the signal sequence so as to render interaction with both the PrlA wild-type protein and the PrlA4 mutant protein less detrimental to the export mechanism. These findings also provide an explanation for the difficulties encountered in **cloning streptokinase** genes in E. **coli** plasmids and maintaining their structural stability.

L26 ANSWER 63 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1988:487410 HCAPLUS
DOCUMENT NUMBER: 109:87410
TITLE: Methylotrophic yeast as vehicles for heterologous gene **expression**
AUTHOR(S): Stroman, D. W.; Hagenson, M. J.
CORPORATE SOURCE: Phillips Res. Cent., Phillips Pet. Co., OK, USA
SOURCE: DECHEMA Monographien (1987), 105(Physiol. Genet. Modulation Prod. Form.), 141-6
CODEN: DMDGAG; ISSN: 0070-315X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The methylotrophic yeast, Pichia pastoris, has been developed as a superior **recombinant** DNA (rDNA) production host. The key component in the development of this host was the **cloning** of the alc. oxidase gene and use of its promoter-regulatory region to control gene **expression**. Heterologous **expression** of several foreign genes in this yeast has been studied. The promoter-regulatory region from the alc. oxidase gene permits very high per cell levels of gene **expression** in an easily regulated manner. These high per cell levels of **expression** can be combined with high cell d. fermentation technol. to yield very high per L production of rDNA products. This is shown by the high levels of production of **streptokinase** in this yeast.

L26 ANSWER 64 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1987:472094 HCAPLUS
DOCUMENT NUMBER: 107:72094
TITLE: New **cloning** vectors for Escherichia **coli** and Bacillus subtilis

INVENTOR(S): Klessen, Christian; Malke, Horst
PATENT ASSIGNEE(S): Akademie der Wissenschaften der DDR, Ger. Dem. Rep.
SOURCE: Ger. (East), 7 pp.
CODEN: GEXXA8
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DD 231083	A1	19851218	DD 1984-264255	19840619
PRIORITY APPLN. INFO.:			DD 1984-264255	19840619

AB New **cloning** vectors for Escherichia **coli** and Bacillus subtilis, derived from the bifunctional plasmid pGR71 by insertion of a promoter-containing DNA fragment into the unique HindIII site of pGR71 upstream of the chloramphenicol acetyltransferase gene, are described. The new plasmids pSM1711, pSM7711, and pSM7712 contain a Streptococcus promoter from plasmids pMF1, pSM10, or pSM7.

L26 ANSWER 65 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1986:509637 HCAPLUS
DOCUMENT NUMBER: 105:109637
TITLE: The **streptokinase** gene
AUTHOR(S): Malke, H.; Ferretti, J. J.
CORPORATE SOURCE: Cent. Inst. Microbiol. Exp. Ther., Ger. Acad. Sci., Jena, DDR-6900, Ger. Dem. Rep.
SOURCE: Folia Haematologica (Leipzig) (1986), 113(1-2), 88-98
CODEN: FOHEAW; ISSN: 0323-4347
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Streptococcus **equisimilis** Gene **skc** for **streptokinase** [9002-01-1] was **cloned** on the vector L47 and then subcloned into plasmids pACYC184 and pBR322 to form **recombinant** plasmids pMF2 (10.4 kb) and pMF5 (9.9 kb), resp., for **expression** in Escherichia **coli**. Plasmid pMF5 contained a 2568-base-pair (bp) insert that included the 1320-bp coding sequence for prestreptokinase. The prestreptokinase comprised 440 amino acid residues, including a 26-amino acid signal peptide. The insert also contained the **skc** upstream regions involved in the regulation of transcription and translation and a 15-bp repeat located 34 bp downstream of the **skc** translation stop signal, which very likely represents the rho-independent transcription terminator. The **skc** gene showed no extended regions homologous to the staphylokinase gene. Heterologous **skc** gene **expression** was also attained in S. sanguis after subcloning of the gene of pMF5 onto plasmid pSM7 to form the bifunctional shuttle plasmid pSM752 (13.3 kb). Plasmid pSM752 was not only functional in E. **coli** and S. sanguis, but also in Bacillus subtilis. The **cloned streptokinase expressed** in E. **coli**, S. sanguis, or B. subtilis has the same specificity as that of the donor strain.

L26 ANSWER 66 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1986:473709 HCAPLUS
DOCUMENT NUMBER: 105:73709
TITLE: **Cloning** of streptococcal genes with Streptococcus-Escherichia **coli** shuttle vector pSA3
AUTHOR(S): Dao, M. L.; Ferretti, J. J.
CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, USA
SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis., 9th (1985), Meeting Date 1984, 233-4. Editor(s):

Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi.
Reedbooks: Bracknell, UK.
CODEN: 55BSAN
DOCUMENT TYPE: Conference
LANGUAGE: English
AB A shuttle vector, the chimeric plasmid pSA3, which can replicate in both E. **coli** and S. sanguis, was constructed. Chromosomal DNA from S. mutans was ligated into this plasmid and **cloned** in E. **coli**. Of 472 **clones** tested, 43 **clones** **expressed** S. mutans surface antigens. A **cloned** S. **equisimilis** **streptokinase** [9002-01-1] gene was inserted into plasmid pSA3 and then used to transform E. **coli**, S. sanguis, and S. mutans, all of which **expressed** the **cloned** **streptokinase** gene.

L26 ANSWER 67 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1986:473707 HCAPLUS
DOCUMENT NUMBER: 105:73707
TITLE: **Cloned** **streptokinase** gene from Streptococcus **equisimilis** H46A
AUTHOR(S): Malke, H.; Ferretti, J. J.
CORPORATE SOURCE: Ger. Acad. Sci., Jena, Ger. Dem. Rep.
SOURCE: Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp. Streptococci Streptococcal Dis., 9th (1985), Meeting Date 1984, 221-2. Editor(s): Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi.
Reedbooks: Bracknell, UK.
CODEN: 55BSAN
DOCUMENT TYPE: Conference
LANGUAGE: English
AB The **streptokinase** [9002-01-1] gene **skc** of S. **equisimilis** was **cloned** in Escherichia **coli** with plasmid pBR322. **Expression** of gene **skc** was observed with both orientations of the gene, which indicated that its own promoter was present and was functional in E. **coli**. **Streptokinase** was excreted by the E. **coli** host. The gene contained a 1320-base-pair open reading frame which encodes 440 amino acids, including a signal peptide of 26 amino acids.

L26 ANSWER 68 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1986:455636 HCAPLUS
DOCUMENT NUMBER: 105:55636
TITLE: The **streptokinase** gene: **cloning**, sequencing and **expression** in new hosts
AUTHOR(S): Malke, Horst
CORPORATE SOURCE: Zentralinst. Mikrobiol., Dtsch. Akad. Wiss., Jena, Ger. Dem. Rep.
SOURCE: Zeitschrift fuer Klinische Medizin (1985) (1986), 41(7), 502-4
CODEN: ZKMEEF; ISSN: 0233-1608
DOCUMENT TYPE: Journal
LANGUAGE: German
AB The **streptokinase** (I) [9002-01-1] gene (**skc**) of Streptococcus **equisimilis** H46A was **cloned** in Escherichia **coli** using vector λL47. One of the **recombinant** **clones** was used to subclone **skc** in E. **coli** plasmid vectors. Plasmids pMF2 (10.4 kilobases, composed of pACYC184 plus a 6.4-kilobase EcoRI fragment) and pMF5 (6.9 kilobases, with a 2.5-kilobase fragment in the PstI site of pBR322) determined I formation in E. **coli**; **expression** of **skc** was independent of its orientation, indicating that the complete gene, together with its control elements, was present. The 2.5-kilobase PstI fragment of pMF5 was isolated and sequenced in the M13 system. Of 2568 base pairs, the largest open reading frame consisted of 1320 base pairs

coding for prestreptokinase, corresponding to I plus its 26-amino acid leader sequence. **Expression** of **skc** was attained in S. sanguis after transformation with the shuttle vector pSM752. In fermentation expts., I production rates of 1500 U/mL were attained, which was below the levels obtained with S. **equisimilis**. Use of pSM752 for similar transformation of Bacillus subtilis is briefly discussed.

L26 ANSWER 69 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1985:608113 HCAPLUS
DOCUMENT NUMBER: 103:208113
TITLE: **Streptokinase**-coding **recombinant** vectors
INVENTOR(S): Ferretti, Joseph J.; Malke, Horst
PATENT ASSIGNEE(S): Phillips Petroleum Co. , USA
SOURCE: Eur. Pat. Appl., 21 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 151337	A2	19850814	EP 1984-306851	19841008
EP 151337	A3	19861008		
R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE				
DD 249037	A1	19870826	DD 1983-255523	19831010
US 4764469	A	19880816	US 1984-585417	19840302
AU 8433859	A1	19850418	AU 1984-33859	19841005
AU 561372	B2	19870507		
ZA 8407873	A	19850529	ZA 1984-7873	19841008
AT 61816	E	19910415	AT 1984-306851	19841008
FI 8403963	A	19850411	FI 1984-3963	19841009
NO 8404039	A	19850411	NO 1984-4039	19841009
DK 8404822	A	19850426	DK 1984-4822	19841009
JP 60237995	A2	19851126	JP 1984-212403	19841009
ES 536623	A1	19870116	ES 1984-536623	19841009
CA 1223223	A1	19870623	CA 1984-464939	19841009
DD 273284	A5	19891108	DD 1984-268254	19841010
US 5066589	A	19911119	US 1988-212254	19880627
US 5187098	A	19930216	US 1992-888420	19920522

PRIORITY APPLN. INFO.:

DD 1983-255523	A	19831010
US 1984-585417	A	19840302
EP 1984-306851	A	19841008
US 1988-212254	A2	19880627
US 1989-348206	B1	19890509

AB **Recombinant** vectors that code for **streptokinase** [9002-01-1] are constructed and **cloned** in Escherichia **coli**. Thus, DNA from Streptococcus **equisimilis** was isolated and digested with the restriction endonuclease Sau3A. DNA fragments of between 4-15 kb were **cloned** into phage λ L47. The ligated phage was infectively added to E. **coli** lawns and **streptokinase**-producing **clones** were isolated. The DNA from one such **clone**, λ L47E **skc** was partially digested with HindIII and then inserted into the HindIII site of plasmid pBR322. The **recombinant** plasmids were used to transform E. **coli** strain HB101. The plasmid isolated from 1 **streptokinase**-producing strain, pMF1, was isolated and a restriction map was prepared A nucleotide sequence anal. of pMF1 showed that the **cloned** fragment encoded for **streptokinase** as well as an amino-terminal signal peptide which is bound to **streptokinase** and which is hydrolyzed during a **streptokinase** secretion event.

L26 ANSWER 70 OF 71 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1985:107414 HCAPLUS
DOCUMENT NUMBER: 102:107414
TITLE: Streptococcus-Escherichia **coli** shuttle vector pSA3 and its use in the **cloning** of streptococcal genes
AUTHOR(S): Dao, My Lien; Ferretti, Joseph J.
CORPORATE SOURCE: Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, 73190, USA
SOURCE: Applied and Environmental Microbiology (1985), 49(1), 115-19
CODEN: AEMIDF; ISSN: 0099-2240
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A shuttle vector that can replicate in both Streptococcus and E. **coli** was constructed by joining the E. **coli** plasmid pACYC184 (chloramphenicol [56-75-7] and tetracycline [60-54-8] resistance) to the streptococcal plasmid pGB305 (erythromycin [114-07-8] resistance). The resulting chimeric plasmid is designated pSA3 (chloramphenicol, erythromycin, and tetracycline resistance) and had 7 unique restriction sites: EcoRI, EcoRV, BamHI, SalI, XbaI, NruI, and SphI. Mol. **cloning** into the EcoRI or EcoRV site results in inactivation of chloramphenicol resistance, and **cloning** into the BamHI, SalI site results in inactivation of tetracycline resistance in E. **coli**. Plasmid pSA3 was transformed and was stable in S. sanguis and S. mutans in the presence of erythromycin. Plasmid pSA3 was used to construct a library of the S. mutans GS5 genome in E. **coli**, and **expression** of surface antigens in this heterologous host was confirmed with S. mutans antiserum. A previously **cloned** determinant that species **streptokinase** [9002-01-1] was subcloned into pSA3, and this **recombinant** plasmid was stable in the presence of a selective pressure and **expressed** **streptokinase** activity in E. **coli**, S. sanguis, and S. mutans.

L26 ANSWER 71 OF 71 LIFESCI COPYRIGHT 2005 CSA on STN
ACCESSION NUMBER: 96:44932 LIFESCI
TITLE: Functional analysis of a relA/spoT gene homolog from Streptococcus **equisimilis**
AUTHOR: Mechold, U.; Cashel, M.; Steiner, K.; Gentry, D.; Malke, H.
CORPORATE SOURCE: Inst. Molecular Biol., Jena Univ., Winzerlaer Str. 10, D-07745 Jena, Germany
SOURCE: J. BACTERIOL., (1996) vol. 178, no. 5, pp. 1404-1411. ISSN: 0021-9193.
DOCUMENT TYPE: Journal
FILE SEGMENT: J; G
LANGUAGE: English
SUMMARY LANGUAGE: English
AB We examined the functional attributes of a gene encountered by sequencing the **streptokinase** gene region of Streptococcus **equisimilis** H46A. This gene, originally called rel, here termed rel sub( )S. **equisimilis**, is homologous to two related Escherichia **coli** genes, spoT and relA, that function in the metabolism of guanosine 5',3'-polyphosphates [(p)ppGpp]. Studies with a variety of E. **coli** mutants led us to deduce that the highly **expressed** rel sub( )S. **equisimilis** gene encodes a strong (p)ppGppase and a weaker (p)ppGpp synthetic activity, much like the spoT gene, with a net effect favoring degradation and no complementation of the absence of the relA gene. We verified that the Rel sub( )S. **equisimilis** protein, purified from an E. **coli** relA spoT double mutant, catalyzed a manganese-activated (p)ppGpp 3'-pyrophosphohydrolase reaction similar to that of the SpoT enzyme. This Rel sub( )S. **equisimilis** protein preparation also weakly catalyzed a ribosome-independent synthesis of (p)ppGpp by an ATP to GTP

3'-pyrophosphoryltransferase reaction when degradation was restricted by the absence of manganese ions. An analogous activity has been deduced for the SpoT protein from genetic evidence. In addition, the Rel sub( )S. **equisimilis** protein displays immunological cross-reactivity with polyclonal antibodies specific for SpoT but not for RelA. Despite assignment of rel sub( )S. **equisimilis** gene function in E. **coli** as being similar to that of the native spoT gene, disruptions of rel sub( )S. **equisimilis** in S. **equisimilis** abolish the parental (p)ppGpp accumulation response to amino acid starvation in a manner expected for relA mutants rather than spoT mutants.

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     (FILE 'HOME' ENTERED AT 17:11:46 ON 29 DEC 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005
L1         42118 S STREPTOKINASE?
L2           630 S "SKC" OR "SKC-2"
L3         42539 S L1 OR L2
L4       7454183 S CLON? OR EXPRESS? OR RECOMBINANT
L5          4154 S L3 AND L4
L6          1362 S EQUISIMILIS
L7           220 S L5 AND L6
L8         49433 S INCLUSION (W) BOD?
L9             5 S L7 AND L8
L10            1 DUP REM L9 (4 DUPLICATES REMOVED)
L11          106 DUP REM L7 (114 DUPLICATES REMOVED)
L12            5 S LAMNDA
L13            1 S L11 AND INCLUSION
L14            0 S L11 AND AGGREGAT?
L15            0 S L11 AND INSOLUBLE
L16            1 S L11 AND SOLUBL?
L17           71 S L11 AND COLI
                 E KUPPUSAMY M/AU
L18           40 S E3
                 E ELLA K/AU
                 E KHATRI G S/AU
L19           46 S E3
                 E LAHIRI S/AU
L20         1660 S E3
                 E SRINIVAS V K/AU
L21           28 S E3
L22         1842 S L17 OR L18 OR L19 OR L20 OR L21
L23           71 S L3 AND L22
L24            1 S L8 AND L23
L25           71 S L4 AND L23
L26           71 S L6 AND L25

=> s l26 and l8
L27            1 L26 AND L8

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L27  ANSWER 1 OF 1  BIOSIS  COPYRIGHT (c) 2005 The Thomson Corporation  on STN
ACCESSION NUMBER:   2000:96119  BIOSIS
DOCUMENT NUMBER:    PREV200000096119
TITLE:              Two streptokinase genes are expressed
                    with different solubility in Escherichia coli
                    W3110.
AUTHOR(S):          Pupo, Elder [Reprint author]; Baghbaderani, Behnam A.;
                    Lugo, Victoria; Fernandez, Julio; Paez, Rolando; Torrens,
                    Isis
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CORPORATE SOURCE: Biopharmaceutical Development Division, Center for Genetic Engineering and Biotechnology, Havana, Cuba
SOURCE: Biotechnology Letters, (Dec., 1999) Vol. 21, No. 12, pp. 1119-1123. print.
CODEN: BILED3. ISSN: 0141-5492.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 15 Mar 2000
Last Updated on STN: 3 Jan 2002

AB The **streptokinase** (SK) gene from S. **equisimilis** H46A (ATCC 12449) was **cloned** in E. **coli** W3110 under the control of the tryptophan promoter. The **recombinant** SK, which represented 15% of total cell protein content, was found in the soluble fraction of disrupted cells. The solubility of this SK notably differed from that of the product of the SK gene from S. **equisimilis** (ATCC 9542) which had been **cloned** in E. **coli** W3110 by using similar **expression** vector and cell growth conditions, and occurred in the form of **inclusion bodies**.

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(FILE 'HOME' ENTERED AT 17:11:46 ON 29 DEC 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 17:12:17 ON 29 DEC 2005

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L1        42118 S STREPTOKINASE?
L2          630 S "SKC" OR "SKC-2"
L3        42539 S L1 OR L2
L4      7454183 S CLON? OR EXPRESS? OR RECOMBINANT
L5         4154 S L3 AND L4
L6         1362 S EQUISIMILIS
L7          220 S L5 AND L6
L8        49433 S INCLUSION (W) BOD?
L9            5 S L7 AND L8
L10           1 DUP REM L9 (4 DUPLICATES REMOVED)
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L12           5 S LAMNDA
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L15           0 S L11 AND INSOLUBLE
L16           1 S L11 AND SOLUBL?
L17          71 S L11 AND COLI
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                E KHATRI G S/AU
L19          46 S E3
                E LAHIRI S/AU
L20        1660 S E3
                E SRINIVAS V K/AU
L21          28 S E3
L22        1842 S L17 OR L18 OR L19 OR L20 OR L21
L23          71 S L3 AND L22
L24           1 S L8 AND L23
L25          71 S L4 AND L23
L26          71 S L6 AND L25
L27           1 S L26 AND L8
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